Compound Discoverer 3.3 SP1 Stable Isotope Labeling Tutorial

To familiarize yourself with using the Thermo Scientific[™] Compound Discoverer[™] 3.3 SP1 application to detect compounds labeled with a stable isotope such as carbon-13, follow the topics in this tutorial. These topics show you how to set up a study and an analysis, process a set of example Xcalibur[™] RAW files, review the result file produced by the analysis, and export the results to a Microsoft[™] Excel[™] spreadsheet.

Note For isotopic labeling experiments, you must use a high resolution accurate mass (HRAM) Thermo Scientific mass spectrometer coupled with a liquid chromatography (LC) inlet to acquire the raw data.

Contents

- Overview
- Start the application
- Check whether the data processing computer can access the external databases
- Set up a new study and a new analysis
- Submit the analysis to the job queue
- Review the analysis results
- Export the analysis results

Overview Before begin this tutorial, locate the example files for a stable isotope labeling study and review the tutorial workflow:

- Locate the example files for this tutorial
- Tutorial workflow
- Use the Help system, the user guides, and the tutorials as needed

In the Compound Discoverer application, data processing takes place within the study environment. To create a practice study, use the example Xcalibur RAW files that are provided in the following folder on the key-shaped USB drive in the software media kit:

Example Studies\LC\Stable Isotope Labeling

To save space on your data processing computer, you can leave the raw data files on the USB drive provided in the software media.

If you do not have the key-shaped USB drive that comes with the media kit, download the example files from the LSMS Software Download and Licensing Portal.

To download the example files

1. Go to the following URL: thermo.flexnetoperations.com

The LSMS Software Download and Licensing Portal website opens.

- 2. Log in.
- 3. Under Software & Services at the left, click the Product List link.
- 4. On the Product List page, click the Application Compound Discoverer link.
- 5. On the Product Information page, click the Compound Discoverer 3.3 SP1 link.

The Product Information page for the Compound Discoverer 3.3 SP1 application contains compressed folders for all the tutorials provided with the application.

Locate the

this tutorial

example files for

thermo scientific

6. On the Product Download Compound Discoverer 3.3 SP1 page, click the file names of the compressed folders (.zip) that contain the example files of interest.

File name	File type	File name	File type
Blank_01.raw	RAW file	Ecoli_12C_AcquireX_ID_01.raw	RAW file
Ecoli_12C_01.raw	RAW file	Ecoli_12C_AcquireX_ID_02.raw	RAW file
Ecoli_12C_02.raw	RAW file	Ecoli_12C_AcquireX_ID_03.raw	RAW file
Ecoli_12C_03.raw	RAW file	Ecoli_12C_AcquireX_ID_04.raw	RAW file
Ecoli_13C_01.raw	RAW file	Stable Isotope Labeling.cdResult	Result file (analysis result)
Ecoli_13C_02.raw	RAW file	Stable Isotope Labeling.cdStudy	Study file
Ecoli_13C_03.raw	RAW file		

7. Copy the files to a folder on your data processing computer.

IMPORTANT For optimal performance, all Compound Discoverer study files (.cdStudy) and result files (.cdResult) should be located on a local hard drive, ideally a solid state drive (SSD). Latency, read- and write speeds of external USB-connected hard drives and network drives are typically much slower than internal hard drives.

Because the result files are continuously accessed throughout the entire data processing workflow, processing times can be significantly longer when using external drives. The RAW files however, are read only once, at the very beginning of the processing workflow and can be located on an internal or external drive.

Tutorial workflow

The typical workflow for a stable isotope labeling analysis includes the following steps.

Step		Task
1	Ø	Start the Compound Discoverer application.
2		Check the computer's access to the mzCloud [™] and ChemSpider [™] databases.
3		Use the New Study and Analysis Wizard to do the following:
5	0	a. Select the study type, create a new study, and select a processing workflow.
	ř.	b. Add the files that you want to process to the study.
		c. Define the sample types for the sample set.
		d. Set up the sample groups for the analysis.
4		Confirm the analysis and start the run.
5	0	Open the result file and review, filter, and sort the data.
6		Sort and filter the data. Then, export the data to a spreadsheet.

Use the Help system, the user guides, and the tutorials as needed

The application provides Help for the views, pages, and dialog boxes. It also provides two user guides—one for LC studies and one for GC studies, six tutorials for various fields of study, and a quick start guide for creating reports with the application's reporting tool.

 Table 1.
 Instructions for accessing the Help, the tutorials, and the user guides

Task	Procedure		
Open the Help topic for a	1. Open the view, page, or dialog box.		
specific view, page, or dialog box	2. On the computer keyboard, press the F1 key or the function key on your keyboard that you have assigned as the Help key.		
	The Help system opens to the topic for the current view, page, or dialog box.		
Find a topic in the Help system for a specific topic or	1. On the Search page in the left pane of the Help system, enter the topic name or the phrase in quotes in the search box.		
phrase	Click List Topics.		
	3. In the Select Topic to Display list, select the topic of interest and click Display .		
Access the manuals as PDF files	From the menu bar, choose Help > Manuals .		
	The following list appears:		
	Compound Discoverer Metabolism Tutorial		
	Compound Discoverer Metabolomics Tutorial		
	• Compound Discoverer E & L Tutorial		
	Compound Discoverer Stable Isotope Labeling Tutorial		
	Compound Discoverer GC EI Tutorial		
	Compound Discoverer GC PCI Tutorial		
	Compound Discoverer User Guide for LC Studies		
	Compound Discoverer User Guide for GC Studies		

• Compound Discoverer Reporting Quick Start





Check whether the data processing computer can access the external databases

Set up a new study and a new analysis To use any of the processing workflows that use the online databases, such as mzCloud[™] and ChemSpider[™], your data processing computer must have unblocked access to these databases on the Internet.

- * To verify that your computer has access to the external mass spectral databases
- 1. From the menu bar, choose Help > Communication Tests.
- 2. Click the mzCloud tab and click Run Tests. When the tests are complete, go to the next step.
- 3. Click the **ChemSpider** tab and click **Run Tests**.
- 4. If your computer has an Internet connection, but these tests fail, leave the Communication Test dialog box open and press the **F1** key to open the Help. Then, follow the instructions to troubleshoot the communication failure.

Go to the next topic to "Set up a new study and a new analysis."

Make sure to copy the example files to an appropriate folder on your data processing computer. See "Locate the example files for this tutorial" on page 1.

Follow these steps to create a new study and a new analysis in the order listed:

- 1. Open the New Study and Analysis Wizard
- 2. Select the study type, specify the directory folder, and name the new study
- 3. Select the processing workflow
- 4. Add the input files to the study
- 5. Specify the sample types
- 6. Set up the sample groups
- 7. Customize the processing workflow

Open the New Study and Analysis Wizard

In the Compound Discoverer application, you use the New Study and Analysis Wizard to create new studies and set up new analyses.

Note After you create a new study and assign sample types to the input files, you can set up different analyses from within the study.

To open the New Study and Analysis Wizard

Do one of the following:

- From the menu bar, choose File > New Study and Analysis.
- From the application toolbar, click the Create a New Study and Analysis from Scratch icon, 2.
- On the Start Page, click the New Study and Analysis link in the What Would You Like to Do? area.

The New Study and Analysis Wizard opens to the Study Name and Processing Workflow page. The first time you create a new study, the (top-level) studies folder is undefined. See Figure 1.

Figure 1. Study Name and Processing Workflow page of the wizard

New Study and Analysis W	izard - Step 1 of 5	-		×	
tudy Name and Processin Specify a unique name fo workflow for the current a	g Workflow r this study and its folder, select the studies folder for storing all of your study folders, and analysis.	select a p	processing	9	
Study Type				-	
Study Name and Directory	y Structure				
Study Name: Studies Folder:	New Study			-	Undefine
Study Template File: Description:	(Optional)				studies i
Processing	(All merun)				
Workflow:	(empty workflow)		×]	
2		1			

Leave this page of the wizard open and go to the next topic to "Select the study type, specify the directory folder, and name the new study."

* To select the study type, specify the directory folder, and name the new study

1. In the Study Type area on the Study Name and Processing Workflow page of the wizard (Figure 1), select the LC option if it is not already selected.

The application stores this selection until you change it.

Note There are two types of studies: GC for gas chromatography-mass spectrometry data and LC for liquid chromatography-mass spectrometry data.

- 2. In the Study Name and Directory Structure area, select the studies directory folder as follows:
 - a. Click the **browse** icon,, next to the Studies Folder box.

The Select Folder dialog box opens.

Select the study type, specify the directory folder, and name the new study b. Browse to the directory where you want to store your studies.

IMPORTANT To avoid excessive processing times, select a directory on your data processing computer. Do not store your studies on an external hard drive.

You can archive your studies on an external hard drive. But, if you need to fully reprocess any of the result files in these studies, move the studies back to a computer that has an installation of the Compound Discoverer application.

During data processing, the application makes a copy of the spectral data in the raw data files and copies this data to the result file, which is located in the folder that has the same name as the study. This processing step is relatively fast, so storing the raw data files on an external hard drive instead of the processing computer does not add a significant amount of time to data processing.

- c. Click New Folder.
- d. Name the new folder Studies, select it, and then click Select Folder (Figure 2).

Figure 2. Select Folder dialog box

Select Folder						×
\leftarrow \rightarrow \checkmark \Uparrow 🖆 \diamond This	PC → OSDisk (C:)		ٽ ~	🔎 Search OSDi	isk (C:)	
Organize 🔻 New folder						?
This PC	^	Name Xcalibur	ĩ	Type File folder File folder	Size	^
Documents Browse for	v or Study Directory.	<	ħ.			>
Folder:	Studies					
				Select Folder	Cancel	
				Select F	older butto	on

Note The first time you create a new study, you must specify the directory (Studies Folder) where you want to store your studies. Thereafter, you can use the same studies folder or create additional studies folders.

3. In the Study Name and Directory Structure area, name the new study in the Study Name box.

For example, type **Stable Isotope Labeling** in the Study Name box.

Note When you create a new study, the application creates a new study folder with the same name and stores the study file (.cdStudy) in the new folder and the new study folder in the specified top-level folder for your studies.

> This PC > OSDisk (C:) > Studies > Stable Isotope Labeling > Stable Isotope Labeling.cdStudy

Leave this page of the wizard open and go the next topic to "Select the processing workflow."

In the Compound Discoverer application, the processing method that interprets the raw data is called a processing workflow (.cdProcessingWF). The application provides defined processing workflows for several applications including stable isotope labeling experiments.

This tutorial uses a defined processing workflow that searches the mzCloud and ChemSpider databases to identify the unlabeled compounds detected in the sample files. It uses the Analyze Labeled Compounds node to detect the isotopologues of these compounds. This workflow also maps compounds to their biological pathways by using the local Metabolika pathway files.

Note If your processing computer does not have Internet access, select the following processing workflow: Stable Isotope Labeling w Metabolika Pathways and ID using Offline Databases.

Select the processing workflow

To select the processing workflow

1. In the Processing area on the Study Name and Processing Workflow page of the wizard, select the following processing workflow from the Workflow list:

Workflow Templates \LC\ Stable Isotope Labeling\Stable Isotope Labeling w Metabolika Pathways and ID using Online Databases

Figure 3. Selecting the processing workflow template from the Workflows list

Jdy Name and Processin Specify a unique name for	or this study and its folder, select the studies folder for storing all of your study folders, and select a processing workflow for the current analysi
Study Type	
	21 💿 29 🔾
Study Name and Director	ry Structure
Study Name:	Stable Isotope Labeling
Studies Folder:	C:\ Studies
Study Template File:	(Optional)
Description:	(Optional)
Processing	
Processing Workflow:	(empty workflow)
Processing Workflow:	(empty workflow) WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mit
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and m: WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal
Processing Workflow:	(empty workflow) WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ Metabolomics \ Untargeted ID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ Metabolomics \ Metabolomics Scoring with Background Removal WorkflowTemplates \ LC \ Metabolomics \ Metabolomics Score Removal WorkflowTemplates \ LC \ Metabolomics \
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w MMDF and Background Removal
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w MMDF and Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w MMDF and Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w MMDF and Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w FISh Scoring and Background Removal
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w MDF and Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ MetID v Natta Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ MetID v Natta Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ NetID v Natta Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ NattiD x Tats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ NattiD x Tats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ NattiD x Tats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ Met
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ MetD \ MetD Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetD \ MetD Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetD \ MetD Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetD \ MetD WatD Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetD \ MetD WatD Stats Expected and Unknown w MMDF and Background Removal WorkflowTemplates \ LC \ MetD \ MetD WatD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatID w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetD \ MetD WatID w Stats Expected wasckground Removal WorkflowTemplates \ LC \ MetID \ MetID waschground \ Natural Product
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID wstats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID wstats Expected and Unknown w MMDF and Background Removal WorkflowTemplates \ LC \ MetID \ MetID wstats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ MetID wstats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ MetID \ MetID wstats Expected W FISh Scoring and Background Removal WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Online and Local Database Searches WorkflowTemplates \ LC \ Neturol \ Vnknown Polymer ID w Stats Online and Local Database Searches WorkflowTemplates \ LC \ OvermetID \ Unknown Polymer ID w Stats Online and Local Database Searches
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Local Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID To WeID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Online and Local Database Searches WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Stats
Processing Workflow:	(empty workflow) WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics using Online Databases, mzLogic, and Molecular Networks WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID Using Local Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases WorkflowTemplates \ LC \ Metabolomics \ Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mz WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Neg Mode WorkflowTemplates \ LC \ MetID \ MetID Generate Inclusion List For Acquisition Pos Mode WorkflowTemplates \ LC \ MetID \ MetID Pattern Scoring with Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected and Unknown w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w Background Removal WorkflowTemplates \ LC \ MetID \ MetID w Stats Expected w FISh Scoring and Background Removal WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Online and Local Database Searches WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Stats Conline and Local Database Searches WorkflowTemplates \ LC \ NaturalProduct \ Natural Product Unknown ID w Stats Online and Local Database Searches

A description of the processing workflow appears in the Workflow Description box.

Workflow:	WorkflowTemplates \ LC \ StableIsotopeLabeling \ Stable Isotope Labeling w Metabolika Pathways and ID using Online Databases 🝸
Vorkflow Description:	Stable Isotope Labeling workflow (untargeted): Using one or more unlabeled reference samples, the application automatically detects all compounds, determines their elemental composition, and identifies the labeled counterparts of these compounds in the samples marked as Labeled. You can use any isotopic label, but you must identify any label other than carbon-13 in the Analyze Labeled Compounds node. The application reports the fractional label incorporation (exchange rate) after natural abundance correction for each compound. Performs retention time alignment, unknown compound detection, and compound grouping across all samples. Predicts elemental compositions for all compounds and hides chemical background (using Blank samples). Detects labeled compounds and reports the fractional label incorporation (exchange rate). Identifies compounds using mzCloud (ddMS2) and ChemSpider (formula or exact mass). Also performs similarity search for all compounds with ddMS2 data using mzCloud. Maps compounds to biological pathways using Metabolika.

2. Read the description.

Note When you complete the wizard, the application creates the Stable Isotope Labeling Examplel.cdStudy file, stores the study file in the Stable Isotope Labeling Example folder, and stores the Stable Isotope Labeling Example folder in the Studies folder.

When you run the analysis in this tutorial, the application stores the result file (.cdResult) in the Stable Isotope Labeling Tutorial folder.

Go to the next topic to "Add the input files to the study."

To add input files to the study

- 1. At the bottom of the Study Name and Processing Workflow page of the wizard, click Next.
 - The Input File Selection page opens.
- 2. On the Input File Selection page, click Add Files.
- 3. In the Add Files dialog box, browse to the folder where you copied the example RAW files.

Tip The application assigns a file number to each input file in the order you import them (see Figure 5). The file numbers are useful for tracking the input files in the result file tables.

4. Select all 11 Xcalibur RAW files in this folder and click Open.

Figure 4. Imported example files

Wew Study and Analysis Wizard - Step 2 of 5	- 🗆 X
Input File Selection Select the input files for this analysis.	
Add Files 🛛 👗 Remove Files	
Files	
Blank_01 D	ate modified: 4/25/2018 10:25:56 PM
Type: RAW File	ize: 202.99 MB
Ecoli_12C_01 D	ate modified: 4/26/2018 3:32:34 AM
Type: RAW File Si	ize: 189.64 MB
Ecoli_12C_02 D	ate modified: 4/26/2018 4:29:13 AM
Type: RAW File	ize: 188.39 MB
Ecoli_12C_03 D	ate modified: 4/26/2018 5:25:52 AM
Type: RAW File	ize: 188.44 MB
Ecoli_12C_AcquireX_ID_01 D	ate modified: 4/25/2018 11:45:14 PM
Type: RAW File Si	ize: 124.89 MB
Ecoli_12C_AcquireX_ID_02 D	ate modified: 4/26/2018 12:04:21 AM
Type: RAW File Si	ize: 123.81 MB
Ecoli_12C_AcquireX_ID_03 D	ate modified: 4/26/2018 12:23:28 AM
Type: RAW File Si	ize: 123.6 MB
Ecoli_12C_AcquireX_ID_04 D	ate modified: 4/26/2018 12:42:35 AM
Type: RAW File Si	ize: 123.27 MB
Ecoli_13C_01 D	ate modified: 4/26/2018 2:17:02 AM
Type: RAW File Si	ize: 188.15 MB
Ecoli_13C_02 D	ate modified: 4/26/2018 5:06:59 AM
Type: RAW File	ize: 188.65 MB
Ecoli_13C_03 D	ate modified: 4/26/2018 5:44:45 AM
Type: RAW File Si	ize: 187.69 MB
11 files	
<i>\$</i>	Cancel < Back Next > Finish

Add the input files to the study

5. Click **Next** to open the Input File Characterization page of the wizard.

Figure 5 shows the newly added samples in the Samples area on the Input File Characterization page of the wizard. By default, the application assigns Sample as the Sample Type to new samples.

Figure 5. Imported files with assigned file numbers

		_		_			
Delimiters: 🗹 U	nderscore Hyphen	Comm	ıa ∐ Sp	bace	Plus 🔄 Other 💦 🌮 Assign 😒 Res	et ा Adva	nced
Study Factors	Paste Copy Add •	Samp	oles				
		Error	Samp 🔺	File		Sample Type	e
							*
			ST	F1	Blank_01	Sample	·
		_	52	F2	Ecoli_12C_01	Sample	
		_	\$3	F3	Ecoli_12C_02	Sample	*
			S4	F4	Ecoli_12C_03	Sample	-
			S5	F5	Ecoli_12C_AcquireX_ID_01	Sample	*
			S6	F6	Ecoli_12C_AcquireX_ID_02	Sample	*
			S7	F7	Ecoli_12C_AcquireX_ID_03	Sample	*
			S8	F8	Ecoli_12C_AcquireX_ID_04	Sample	-
			S9	F9	Ecoli_13C_01	Sample	*
			S10	F10	Ecoli_13C_02	Sample	-
			S11	F11	Ecoli_13C_03	Sample	*
		4			Ш		
			how Acco	cipted	File		

— File numbers based on the import order

Leave this page of the wizard open and go to the next topic to "Specify the sample types."

Specify the sample types

To specify the sample types for the example files in this tutorial, do the following in any order on the Input File Characterization page of the wizard:

- Automatically assign the blank sample type
- Specify the identification samples
- Specify the labeled samples

Table 2 describes the sample types for a stable isotope labeling analysis.

Table 2. Sample types

Sample type	The application processes these sample types as follows
Sample	Detects the unlabeled compounds in the sample.
Blank	Marks the background compounds in the entire data set.
Identification Only	Does not report the chromatographic peak areas for the compounds in these samples. Uses the sample's fragmentation scans for component (compound) identification.
Labeled	Determines the isotopic label incorporation.

Automatically assign the blank sample type

To assign the Blank sample type

Note When you select the appropriate delimiters, the application assigns the Blank sample type to files named Blank or files with Blank in the file name.

In the command bar, click Assign.

🧚 Assign \, 😒 Reset 🛭 🖜 Advanced

The application assigns the Blank sample type to the Blank.raw file.

Go to the next topic to identify the samples to be used to identify the compounds in the unknown samples.

Identification samples must have fragmentation scans. In the example data set, the Acquire_X_ID.raw files contain data-dependent fragmentation scans (acquired within the same acquisition sequence and the same chromatographic conditions as the other data files).

To specify the samples to use for compound identification

Use the SHIFT key to select the four Acquire_X_ID files. Then, right-click the selected rows and choose **Set Sample Type To > Identification Only** (Figure 6).

Tip To select a row, you can click any column but the Sample Type column.

Figure 6. Defining the samples to be used for Identification Only

Τ	S 4	F	F4	Ecoli_12C_03	Sample *
	S5	F	F5	Ecoli_12C_AcquireX_ID_01	Copy With Headers Ctrl+C
	S6	F	F6	Ecoli_12C_AcquireX_ID_02	Copy
	S7	F	F7	Ecoli_12C_AcquireX_ID_03	
	S8	F	F8	Ecoli_12C_AcquireX_ID_04	Clear Selection
	S9	S9 F9 Ecoli_13C_01		Ecoli_13C_01	Cell Selection Mode
	•			III	Enable Row Grouping
	✓ Show	Associ	iated I	File	
					Set Sample Type to Sample
				Cancel < I	Control
					Blank
					Quality Control
					Identification Only
					Standard
					Labeled

Go to the next topic to identify the labeled samples.

Specify the labeled samples

The tutorial data set includes three samples labeled with carbon-13.

To specify the labeled samples for the detection of labeled compounds

Use the CTRL key to select the files with 13C in their file name. Then, right-click the selected rows and choose **Set Sample Type To > Labeled** (see Figure 7 on page 11).

Specify the identification samples

Figure 7. Defining the labeled samples

Stuc	ly Definiti	on	nput Files Samples Analy	sis Results				
rror	Samp 🔺	File	Sample Identifier	Sample Type				
			II		•			
	S1	F1	Blank_01	Blank	*			
	S2	F2	Ecoli_12C_01	Sample	-	Copy With Headers Ctrl-	·c	
	S3	F3	Ecoli_12C_02	Sample	*	Сору		
	S4	F4	Ecoli_12C_03	Sample	*	Class Selection		
	S5	F5	Ecoli_12C_AcquireX_ID_01	Identification Only	*	Clear Selection		
	S6	F6	Ecoli_12C_AcquireX_ID_02	Identification Only	-	Cell Selection Mode		
	S7	F7	Ecoli_12C_AcquireX_ID_03	Identification Only	-	Enable Now Grouping		
	S8	F8	Ecoli_12C_AcquireX_ID_04	Identification Only	-	Set Sample Type to	- F [Sample
	S9	F9	Ecoli_13C_01	Labeled	*			Control
	S10	F10	Ecoli_13C_02	Labeled	*	Set as Input File		Blank
	S11	F11	Ecoli_13C_03	Labeled	*			Quality Control
								Identification Only Standard
								Labeled

Set up the sample groups

To set up the sample groups for the analysis

1. At the bottom of the Input File Characterization page, click Next.

The Sample Groups and Ratios page of the wizard opens. Use this page of the wizard to set up the sample groups and ratios for a differential analysis.

Figure 8 shows the 11 individual example files with their defined sample types in the Generated Sample Groups pane. Because you have not yet selected the study variables, the samples are not grouped.

Figure 8. Generated sample groups without any study variables

Sample Group and Ratio Specification	Generated Sample Groups
- Study Variables	Dirah Et. Dirah 01
	Sample F2: Fael: 12C 01
E File	Sample F2: Ecol_12C_01
Sample Type	Sample F3: ECOI_12C_02
	IdentificationOnly E5: Ecoli 12C AcquireX ID 01
Manual Batia Constantian	IdentificationOnly E6: Ecoli 12C_AcquireX_ID_01
Manual Ratio Generation	IdentificationOnly F7: Ecoli 12C AcquireX ID 03
No sample groups available for creating ratios.	IdentificationOnly F8: Ecoli 12C AcquireX ID 04
······································	Labeled F9: Ecoli 13C 01
	Labeled F10: Ecoli 13C 02
- Bulk Ratio Generation	Labeled F11: Ecoli 13C 03
No sample groups available for creating ratios	Generated Ratios 🕺 Clear A

Tip Unlike a typical metabolic flux experiment, this tutorial does not include time as a study variable. For information on how to set up the study factors for a metabolic flux experiment, follow the embedded wizard Help or press the F1 key to access the Help system.

The time points in a flux experiment are the items for a categorical study factor.

2. In the Study Variables area of the Sample Groups and Ratios page of the wizard, select the **Sample Type** check box.

The sample groups—Blank, Sample, Identification Only, and Labeled—appear in the Generated Sample Groups area (see Figure 9 on page 12).

Note For the example data set, grouping the samples by sample type makes reviewing the data in the result tables easier.

Tip If you are setting up a metabolic flux study for your own data set, use the Sample Groups and Ratio page to set up the ratios for a differential analysis.

Figure 9. Samples grouped by sample type

Wew Study and Analysis Wizard - Step 4 of 5	- 🗆 ×
Sample Groups and Ratios Select the study variables for sample grouping and add ratios for gro	oup comparisons.
Sample Group and Ratio Specification	Generated Sample Groups
Study Variables	Blank Blank F1: Blank_01 IdentificationOnly IdentificationOnly F5: Ecoli_12C_AcquireX_ID_01 IdentificationOnly F6: Ecoli_12C_AcquireX_ID_02 IdentificationOnly F7: Ecoli_12C_AcquireX_ID_03
Numerator: `` Denominator: ``	IdentificationOnly F8: Ecoli_12C_AcquireX_ID_04
Bulk Ratio Generation Denominators to be used: Sample Type : Labeled Sample Type : Sample	Labeled F9: Ecoli_13C_01 Labeled F10: Ecoli_13C_02 Labeled F11: Ecoli_13C_03
	Sample F2: Ecoli_12C_01 Sample F3: Ecoli_12C_02 Sample F4: Ecoli_12C_03
Add Ratios	Generated Ratios 🔀 Clear All
₹	Cancel < Back Next > Finish

3. At the bottom of the Sample Groups and Ratios page, click Finish to save the study and close the wizard.

The study page with its four subpages and the analysis that you set up with the wizard open. See Figure 11.

The Analysis view lists the 11 input files in the example data set. The analysis is set up to combine the processed results from these files into one result file—that is, the By File check box is clear and the file name for the result file is available for editing.

	Tabs for four Study page tab Ana the subpages pag of the study			pages				7 4		V	Vorkflows	s paç
filler for the second s	Start P Add File	Page × rll ₁ Stable Isotope Lates Remove Files & Ope	n Containing Folder	New Analysis	🕼 Open An	alysi	is Ten	nplate	By File	Run	Save	* ×
Error		Name	File Type	Sample Information		٦ŀ	many			Gp Harr	ang ourc	
					-		Prov	reccipo	Step (Fully Processing	,)	Edit	
	F1	Blank 01	raw	Sample Type: [Blank]	1		FIO	lessing	Step (rully Processing	<i>v</i>	- con	
-	F2	Ecoli 12C 01	raw	Sample Type: [Samp	le]		Wo	orkflow	Stable Isotope Label	ling w Met	abolika	
	F3	Ecoli 12C 02	.raw	Sample Type: [Samp	le]		_		Pathways and ID usi	ing Online	Databases	
	F4	Ecoli 12C 03	.raw	Sample Type: [Samp	le]		Re	sult File	Blank_01.cdResult			
	F5	Ecoli 12C AcquireX ID 01	.raw	Sample Type: [Identi	fication Only]		▼	Files fo	or Analysis: (11)		样 Clear A	AII
	F6	Ecoli_12C_AcquireX_ID_02	.raw	Sample Type: [Identi	fication Only]		×	F1	Blank_01	S	ample Type	: [B
	F7	Ecoli_12C_AcquireX_ID_03	.raw	Sample Type: [Identi	fication Only]		×	F2	Ecoli_12C_01	S	ample Type	: [Si
	F8	Ecoli_12C_AcquireX_ID_04	.raw	Sample Type: [Identi	fication Only]		×	F3	Ecoli_12C_02	S	ample Type	: [Si
	F9	Ecoli_13C_01	.raw	Sample Type: [Labele	ed]		×	F4	Ecoli_12C_03	S	ample Type	: [S
	F10	Ecoli_13C_02	.raw	Sample Type: [Labele	ed]		×	F5	Ecoli_12C_AcquireX_I	D_01 S	ample Type	: [Ic
	F11	Ecoli_13C_03	.raw	Sample Type: [Labele	ed]		×	F6	Ecoli_12C_AcquireX_I	D_02 S	ample Type	: [Ic
							×	F7	Ecoli_12C_AcquireX_I	D_03 S	ample Type	: [Ic
							×	F8	Ecoli_12C_AcquireX_I	D_04 S	ample Type	: [Ic
							×	F9	Ecoli_13C_01	S	ample Type	: [Li
							×	F10	Ecoli_13C_02	S	ample Type	: [Li
							×	F11	Ecoli_13C_03	S	ample Type	: [Li
							۹ 🗉					•
S S	how De	etails				1						

Figure 10 shows the Input Files page of the study and the Analysis view with a list of files for analysis. **Figure 10.** Study with an analysis that is ready for processing

Files for analysis

Customize the processing workflow

Before submitting the analysis to the job queue, review the processing workflow and make changes as needed.

* To review and customize the processing workflow for this tutorial

1. Click the **Workflows** tab to open the Workflows page of the analysis.

The Workflows page displays the processing workflow that you selected with the wizard.

Tip You can open the Workflows page in two ways:

- Click the **Workflows** tab to the left of the Analysis view.
- Click **Edit** in the Analysis view to the right of Processing Step.

Figure 11 on page 14 shows the processing workflow in the Workflow Tree pane.



Figure 11. Processing workflow template for a stable isotope labeling experiment

IMPORTANT The minimum peak intensity setting in the Detect Compounds (Legacy) node specifies the minimum height (for the base spectral peak of a chromatographic peak) for detecting and reporting chromatographic peaks.

For this tutorial, change the setting to 100000 (1e5), as the raw data files were acquired with an Orbitrap $ID-X^{m}$ mass spectrometer (see Table 3).

- 2. To customize the minimum peak intensity for the Detect Compounds (Legacy) node, do the following,
 - a. In the Workflow Tree area on the Workflows page, click the Detect Compounds (Legacy) node to select it.

A dashed green border appears around the Detect Compounds (Legacy) node.



In addition, the parameters page for the node opens to the left of the Workflow Tree pane. Most of the parameters for the node are set to their default values, but Thermo Fisher Scientific customized a few of the parameters settings for the processing workflow template.

Figure 12. Parameter settings for the Detect Compounds (Legacy) node

Pa	rameters of 'Detect Compounds (Le	:gacy)'	
Sł	now Advanced Parameters		
~	1. General Settings Mass Tolerance [ppm] Intensity Tolerance [%] Min. Peak Intensity Ions Min. Element Counts Max. Element Counts	5 ppm 30 1000000 [2M+ACN+H]+1; [2M+ACN+Na]+1; [2M+FA-H]-1; C H C 90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5	Thermo Fisher Scientific set this parameter to 1E6 in the processing workflow templates for stable isotope labeling.
M Th ch M M	Use Most Intense Isotope Only in. Peak Intensity is parameter specifies the minimur romatographic peaks in the XIC tra inimum value = 0.0 aximum value = (unchecked) forkflow Nodes Parameters of 'D	False m base peak height to detect and report ces. etect Compounds (Legacy)'	False in the processing workflow templates for stable isotope labeling.

Figure 13. lons list in the Detect Compounds (Legacy) node

✓ 1. General Settings Mass Tolerance [ppm] 5 ppm Intensity Tolerance [%] 30 Min. Peak Intensity 100000 Ions]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1 Min. Element Counts □ Show Checked Only (32/33) Wax. Element Counts □ Show Checked Only (32/33) Use Most Intense Isotope Only Filter ✓ [2M+ACN+H]+1 [2M+FA-H]-1 ☑ [2M+ACN+H]+1 [2M+FA]+1 ☑ [2M+K]+1 [2M+H]+1 ☑ [2M+H]+1 [2M+H]+2 □ [M+2H]+2 [M+4CN+H]+1 ☑ [M+4CN+H]+1 [M+4CN+H]+1 ☑ [M+4CN+H]+1 [M+4CN+H]+1	Show Advanced Parame	eters		
Mass Tolerance [pm] 5 ppm Intensity Tolerance [%] 30 Min. Peak Intensity 100000 Ions]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1 Min. Element Counts Show Checked Only (32/33) Max. Element Counts [Show Checked Only (32/33) Use Most Intense Isotope Only Filter Image: Comparison of Mathematical State (State (Sta	✓ 1. General Setting	gs		
Intensity Tolerance [%] 30 Min. Peak Intensity 100000 Ions]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1 Min. Element Counts] Show Checked Only (32/33) Max. Element Counts [Show Checked Only (32/33) Use Most Intense Isotope Only [Filter Image: Comparison of Max Intense Isotope Only [2M+ACN+H]+1 [2M+ACN+H]+1 [2M+FA-H]-1 [2M+K]+1 [2M+K]+1 [2M+K]+1 [2M+K]+1 [2M+H]+1 [2M+H]+1 [2M+H]+2 [M+ACN+H]+1 [M+ACN+H]+1 [M+ACN+H]+1 [M+ACN+H]+1 [M+ACN+H]+1 [M+ACN+H]+1 [M+C]-1 [M+DNSO+H]+1 [M+C]-1	Mass Tolerance [p	pm]	5 ppm	
Min. Peak Intensity 100000 Ions]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H+H2O]-1 Min. Element Counts	Intensity Tolerance	e [%]	30	
Ions]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H+H2O]-1 Min. Element Counts	Min. Peak Intensit	y	100000	
Min. Element Counts Max. Element Counts Use Most Intense Isotope Only Filter Filter Image: Constant and the provided of the pr	lons		.]-1; [M-H]-1; [M-H+HAc]-1; [M-H+TFA]-1; [M-H-H2O]-1	~
Use Most intense isotope Unity [Inter Image: Construct of the second s	Min. Element Cour Max. Element Cou	nts nts	Show Checked Only (32/33)	5
	Use Most Intense I	sotope Only		_
	lons This parameter allows	selection of multipl		

For the lons parameter, Thermo Fisher Scientific selected 32 out of 33 ions in the processing workflow templates for stable isotope labeling. Notice that the triply charged ion is not selected. b. In the parameters page for the Detect Compounds (Legacy) node, type 1e5 in the Min. Peak Intensity box.

Note The Analyze Labeled Compounds node requires input from the Detect Compounds (Legacy) node.

The Use Most Intense Isotope Only parameter is set to False in the processing workflow templates for stable isotope labeling and 32 out of 33 ion definitions are selected for the Ions parameter.

Table 3 lists the recommended range for the Min. Peak Intensity parameter, which depends on the sensitivity of the mass spectrometer.

Table 3. Recommended minimum peak intensity range

Mass spectrometer	Minimum peak intensity (chromatographic peak height)
Q Exactive™, Q Exactive Plus™, Q Exactive HF	500 000 to 1 000 000
Exactive™, Exactive Plus™, Orbitrap Elite™, Orbitrap Velos Pro™	100 000 to 500 000
Orbitrap Fusion™, Orbitrap Lumos, Orbitrap ID-X	50 000 to 100 000
LTQ Orbitrap XL™, LTQ Orbitrap Velos™	25 000 to 100 000

3. (Optional) To learn more about the parameter settings for the workflow nodes in the processing workflow template for stable isotope labeling studies, review the settings in Table 4.

Table 4. Processing workflow node settings for the stable isotope processing workflow template (Sheet 1 of 2)

Group Compounds node

In the processing workflow template for stable isotope labeling, the RT Tolerance is set to 0.2 min.

The Group Compounds node creates the MSn tree that the analysis sends to the search nodes and saves to the result file. The Predict Compositions node uses the MSn tree to match fragments.

Par	ameters of 'Group Compounds'			
Sh	ow Advanced Parameters			
~	1. General Settings			
	Mass Tolerance	5 ppm		
	RT Tolerance [min]	0.2		
	Align Peaks	False		
	Preferred lons	[M+H]+1; [M-H]-1		
	Area Integration	All lons		
\sim	2. Peak Rating Contributions			
	Area Contribution	3		
	CV Contribution	10		
	FWHM to Base Contribution	5		
	Jaggedness Contribution	5		
	Modality Contribution	5		
	Zig-Zag Index Contribution	5		
~	3. Peak Rating Filter			
	Peak Rating Threshold	0	-	 The peak rating filter
	Number of Files	0		is not enabled.

Table 4. Processing workflow node settings for the stable isotope processing workflow template (Sheet 2 of 2)

Assign Compound Annotations node

For the example data set, do not change the settings.

Parameters of 'Assign Compound Annotations'						
Hic	le Advanced Parameters					
~	1. General Settings					
	Mass Tolerance	5 ppm	-			
~	2. Data Sources					
	Data Source #1	mzCloud Search				
	Data Source #2	Predicted Compositions				
	Data Source #3	MassList Search				
	Data Source #4	ChemSpider Search				
	Data Source #5	Metabolika Search				
	Data Source #6					
	Data Source #7					
\sim	3. Scoring Rules					
	Use mzLogic	True				
	Use Spectral Distance	True				
	SFit Threshold	20				
	SFit Range	20				
\sim	4. Reprocessing					
	Clear Names	False				

Tip If you are working with your own data set and the analysis does not identify the correct isotopologues, consider changing Data Source #1 to a custom mass list for your analytes and reprocessing the analysis.

Analyze Labeled Compounds node

For the example data set, keep the default settings. For a different data set, enter the appropriate isotope for the Label Element parameter.

Parameters of 'Analyze Labeled (Compounds'	
Show Advanced Parameters		
 Label Settings Label Element Max. Exchange 	[13]C	Customize this setting for your own data set, as appropriate. Specifies the maximum number of exchangeable atoms.
Source Efficiency [%] 2. Pattern Analysis	100	The default setting is 25.
Mass Tolerance [ppm] Intensity Tolerance [%] Intensity Threshold [%] S/N Threshold	5 ppm 30 0.1 3	If you set this value to 0, the analysis determines the maximum number of exchangeable atoms for each compound from the compound's elemental composition.
 General Settings Mark Irregular Exchange Exclude Blanks Hide Unprocessed 	True True True	Hides the compounds without formulas in the Compounds table.

Submit the analysis to the job queue

For the example data set and analysis, you can now submit the analysis to the job queue for processing.

Tip If you modified the analysis and the Run button is unavailable, do the following:

- Remedy the issues listed in the Current Workflow Issues pane on the Workflows page.
- If there is a Caution symbol to the right of Edit in the Analysis view, point to it and remedy other analysis errors, for example, no input files in the Files for Analysis area of the Analysis view or missing node connections.

If a prompt appears when you submit the run, open the Grouping & Analysis page of the analysis and select the Sample Type check box to group the input files by sample type.

To submit the analysis to the job queue

1. To create one result file for the input file set, leave the By File check box clear in the Analysis view.

In this analysis (and for most analyses), you compile the processing results from multiple input files into one result file.

Analysis 🗌 By File 💡 Run 📙 Sa	ve X	١
-------------------------------	------	---

By default, the application uses the name of the first input file for the file name of the result file.

2. In the Result File box, rename the result file Stable Isotope Labeling.

Analysis By File	🗊 Run	🔒 Save	×		
Processing Step (Fully Processing)		Edit		t	 Available Run command
Workflow: Stable Isotope Labeling w Metabolika Pathways and ID using Online Databases Result File: Stable Isotope Labeling cdResult					_ Result file name
Resolution stable isotope cabeling/caresolution					

3. Click Run.

The Analysis Validation Issues prompt opens with an alert about the lack of a peak rating filter in the Group Compounds node.

4. Click Ignore.

The Job Queue page opens.

5. To view the processing messages, click the expand icon, \pm , to the left of the job row.

	🟫 Start Page 🗙	III) Stable Isotope	Labeling	× 🗟 Jo	b Queue ×	
	🎲 Abort 🐗 Pro	mote 🐹 Remove	e ಿ Re	efresh 🛛 🕷	Open Results	Display Verbose Messages
Expand	Job Queue:					
icon	Execution Order	Execution State	Details	Progress	Туре	Name
	=					
		Running		12%	Processing	Stable Isotope Labeling

Note During the run, the Search ChemSpider node generates warning messages, which you can ignore. Warning messages have a yellow background.

6. Leave the Job Queue page open and go to "Review the analysis results."

Review the To review the analysis results, follow these topics in the order listed: analysis 1. Open the result file results 2. Review the default layout for the result page and common layout modifications 3. Apply the Stable Isotope Labeling layout 4. Review the exchange rates 5. Review the labeling status 6. View a trend chart for a single compound or a set of trend lines for multiple compounds 7. View the distribution of the isotopologues for each compound 8. View the Metabolika pathways for a compound For more information about a specific result table or view, select the table or view to make it active, and then press the F1 key. The Compound Discoverer application provides F1 Help for all the views that you access from the View menu and all the result tables. **Open the result** You can open a result file from multiple locations: the Job Queue page, the Analysis Results page of a study, the file Compound Discoverer Start Page, or the menu bar. Note For this tutorial, you can create a result file by setting up and running an analysis with the example data set. Or, you can open the result file-Stable Isotope Labeling-in the same folder where you found the example data set.

* To open the result file generated by the analysis

When the application completes the run, double-click the run on the Job Queue page.

/	The Start Page × III Stable Isotope Labeling × 3 Job Queue ×										
1	🎲 Abort 🌼 Promote 🧩 Remove 💐 Refresh 🚯 Open Results 🗌 Display Verbose Messages										
	Job Queue:										
IΓ	Execution Order Execution State Details Progress Type Name										
[±	Completed	Warnings	100%	Processing	Stable Isotope Labeling					

Review the default layout for the result page and common layout modifications

These topics describe the default layout for the result file that the stable isotope labeling analysis generates and some of the common layout modifications that you can make:

- Default layout for the stable isotope labeling analysis
- Common layout modifications

Default layout for the stable isotope labeling analysis The result file opens as a tabbed document with the following layout (numbered in Figure 14 on page 20):

- 1. The Chromatograms view appears on the upper left of the page.
 - The Chromatograms view displays the overlaid and shaded chromatographic peaks for the compound in the first row of the Compounds table.
- 2. The Mass Spectrum view appears on the upper right of the page.

The Mass Spectrum view displays the MS1 scan in the spectrum tree that is closest to the apex of the compound's chromatographic peak.

The spectrum tree to the left of the spectrum plot includes the MS1 scans and the fragmentation scans for the preferred ions that the MS acquired within the following retention time window:

- The chromatographic peak apex for the selected compound ± peak width at half maximum (FWHM) –or–
- The Start and end points of the chromatographic peak, as determined by the peak detection algorithm

Note If the data set does not include data-dependent MS2 scans within the retention time window but does include data-independent scans within this window, the spectrum tree includes the data-independent scans.

3. A set of tabbed result tables opens in the bottom half of the page. The Compounds table is the active table.

Because the selected processing workflow includes the Mark Background Compounds node and the Analyze Labeled Compounds node, the Compounds tab has a filter icon with a check mark (\Im). The compounds that the analysis identified as background compounds are marked as background compounds in both the blank and non-blank samples and are hidden from the table. In addition, the compounds without a formula are hidden from the table.

4. A collapsed area for the related data tables below the main tables.

In the Compounds table, the detected compounds are listed in descending order of the maximum chromatographic peak area [Area (Max.)] across the set of input files. The Chromatograms and Mass Spectrum views are populated with data for the first compound in the table.

Figure 14 shows the factory default layout for the Stable Isotope Labeling.cdResult file.

Figure 14. Default result file layout



Common layout modifications

You can change which columns, tables, and views are visible or hidden. To display the subcolumn headings for columns that contain multiple subcolumns, you must expand the column headers.

 Table 5.
 Common layout modifications

Task	Procedure
Show or hide a table column	Open the Field Chooser for a table by clicking the icon, 🖆, in the upper left corner of the table. To display a column, select its check box. To hide a column, clear its check box.
Show or hide a table	Open the Select Table Visibility dialog box by clicking the icon, ill, at the left of the table tabs.
Close a view	Click the close icon, X , in the upper right corner of the view.
Open a view	In the application menu bar, choose View > <i>Specific View</i> . Or, in the toolbar, click the icon for the view.
Expand the header for a column with multiple subcolumns	Click the expand icon, ⊕, to the right of the heading.
Freeze a column to the left side of the table	Right-click the table and choose Enable Column Fixing . Then, click the pin icon to the right of the column heading.
Pin a row to the top of the result table.	Click the pin icon to the right of the row number (unpinned, \dashv , or pinned, \clubsuit)
Sort a result table by a column with numeric or text information.	Click a column header once or twice to sort the rows in ascending order (\blacktriangle) or descending order (\blacktriangledown) , based on the contents of the column.
Sort a result table by multiple columns.	Click the column header of the primary sort column once or twice to sort the rows in ascending order (\bigstar) or descending order (\blacktriangledown), based on the contents of the column.
	Hold down the CTRL key and click the column header of the secondary sort column once or twice to set the sort order.
Reset the result page layout.	From the application menu bar, choose Window > Reset Layout .

Apply the Stable Isotope Labeling layout

The application comes with the factory default layout and two named layouts: Statistics and Stable Isotope Labeling. When reviewing the results of a stable isotope labeling analysis, apply the Stable Isotope Labeling layout.

To apply the Stable Isotope Labeling layout to the current result file

From the application menu bar, choose **Window > Apply Layout > Stable** Isotope Labeling.

Applying the Stable Isotope Labeling layout does the following to the example result file as shown in Figure 15 on page 23:

- Hides the following main tables:
- Compounds per File - Labeled Compounds per File
- Features per File - Labeled Features
- ChemSpider Results - mzCloud Results
- Hides the following columns in the Compounds table:
- #Adducts
- #Metabolika Pathways
- Annot. Δ Mass [Da]
- Annotation MW
- Avg. Exchange
- Background
- FISh Coverage

- Gap Status
- Metabolika Pathways
- MS Depth
- mzCloud Library Matches
- RT Tolerance [min]
- Structure
- Opens the Related Tables pane to the Labeled Compounds per File table for the first compound in the Compounds table.
- Opens the Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views as tabbed views on the bottom right of the page.
- Selects the Rel. Exchange data property for the Trend Chart view.

Fiel	d Chooser 🛛 🗴
	# Adducts
1	# ChemSpider Results
	# Metabolika Pathways
1	# mzCloud Results
	# Scan IDs (mzCloud)
1	Annot. Source
	Annot. ∆Mass [Da]
4	Annot. ΔMass [ppm]
	Annotation MW
4	Area
1	Area (Max.)
	Avg. Exchange
1	Background
1	Calc. MW
1	Checked
	FISh Coverage
1	Formula
	Gap Status
1	Labeling Status
1	m/z
	Metabolika Pathways
	MS Depth
1	MS2
1	mzCloud Best Match
1	mzCloud Best Match Confidence
	mzCloud Library Matches
1	Name
1	Peak Rating
	PQF: FWHM2Base
	PQF: Jaggedness
	PQF: Modality
	PQF: Zig-Zag Index
	Reference Ion
1	Rel. Exchange [%]
1	RT [min]
	Structure
1	Tags

F





Review the exchange rates

View the relative exchange rates for a compound across the input file set The following topics show you how to modify the layout of the result page for easier visualization of the exchange rate columns in the Compounds table:

- View the relative exchange rates for a compound across the input file set
- View the exchange rate for each of the compound's isotopologues
- View the exchange rates for the adducts of a compound

Precondition: You applied the Stable Isotope Labeling layout to the result file as described in "Apply the Stable Isotope Labeling layout" on page 22.

In this topic, you review the exchange rates for L-glutamic acid. The chromatographic peak for this compound has the second largest maximum area among all the detected compounds, so it sorts to row 2 when the Compounds table is sorted in descending order by the Area (Max). column.

Note The Annotation Source column is hidden in the following figures.

* To view the relative exchange rates for a compound across the input file set

1. In the Compounds table, click the pin icon to the left of L-Glutamic acid in row 2.

Clicking the pin icon to the right of a row number freezes the row to the top of the table.

Figure 16. L-glutamic acid frozen at the top of the Compounds table

Compo	ounds 😵 🛛 Input Files	Study Info	ormation Stat	istical Methods	Metabolika Pathways				
Ē	Name 👳	Checked 🕀	Tags 🛛 🛨 🕀	Formula 🕂	Annot. ∆Mass [ppm] ⊣⊐	Calc. MW 🗠	m/z ∹⊐	RT [min] 🗇	Area (Max.) 🔻 🕀
2 🕂	L-Glutamic acid		00000	C5 H9 N O4	-1.65	147.05291	148.06017	1.287	2076701132
1 👳	L-Glutathione (reduced)		00000	C10 H17 N3 O6 S	-1.71	307.08328	308.09056	2.086	2801461806

- 2. In the Compounds table, freeze the Name column at the left of the table as follows:
 - a. Right-click the Compounds table and choose Enable Column Fixing. See Figure 17.

Figure 17. Shortcut menu for the Compounds table

	Copy With Headers	Ctrl+C
	Сору	
	Copy Structure	•
	Clear Selection	
	Cell Selection Mode	
	Enable Column Fixing	
	Collapse All Column Headers	
	Expand All Column Headers	
	Check Selected	+
	Check All	+
	Uncheck Selected	•
	Uncheck All	•
	Remove All Checkmarks in All Tables	
	Add Tag	+
	Remove Tag	•
	Set Tags	
	Remove All Tags in All Tables	
	Edit Compound Annotation	
	Clear Compound Annotation	
	Apply FISh Scoring	
	Export	+
_		

Pin icons appear to the right of the column headings.



- b. Click the pin icon to the right of the Name column heading (pinned, \square , and unpinned, \square).
- 3. Scroll right to the Rel. Exchange [%] column.
- 4. To view the input file names in the Rel. Exchange [%] column, click the expand icon (⊞) next to the column header. Or, right-click the Compounds table and choose **Expand All Column Headers** to expand all the table column headers.

Figure 18 shows the relative exchange rate for L-glutamic acid in each input file. The relative exchange rate is 98% for the labeled samples and 0% for the unlabeled samples.

Figure 18. Relative exchange rate for L-glutamic acid

2	Job Q	ueue × 🕅 Stable Isotope Labelin	g ×						
	Compo	ounds 😵 🛛 Input Files 🛛 Study Infe	ormation Statistical N	lethods Me	tabolika Pathways				
				Rel. Exchang	je [%]				= +
É	₫	Name 4	.abeling Status 🔹 ≒	coli_13C_01.raw (F9)	coli_13C_02.raw (F10)	coli_13C_03.raw (F11)	coli_12C_01.raw (F2)	coli_12C_02.raw (F3)	coli_12C_03.raw (F4)
2	џ.	L-Glutamic acid		98	98	98	0	0	0
1	-12	L-Glutathione (reduced)		98	98	98	0	0	0
3	÷Þ			26	13	15	26	15	15
4	÷12			44	44	44	44	44	44
5	÷Þ	Dodecamethylcyclohexasiloxane		0	0	0	0	0	0
6	÷12	L-Glutathione oxidized		98	98	98	0	0	0
7	-12			10	9	10	9	9	9
8	- =	NP-008095		27	27	27	27	27	27
9	-12	N-Acetylputrescine		94	93	93	0	0	0
4									
 ⊘ s	how R	elated Tables							
/									

Name column frozen at the left of the Compounds table

98% relative exchange rate for L-glutamic acid in the three labeled samples (F9, F10, and F11)

View the exchange rate for each of the compound's isotopologues

To view the exchange rate for each isotopologue of a compound

- 1. In the main Compounds table, select L-glutamic acid (row 2).
 - If you applied the Stable Isotope Labeling layout, the related tables pane is visible and the Labeled Compounds per File table is the active table. See "Apply the Stable Isotope Labeling layout" on page 22.
 - If you fixed the Name column to the left of the table, you can scroll to the right without losing track of which compound you are viewing. See step 2 in "View the relative exchange rates for a compound across the input file set" on page 23.
- 2. Click Show Related Tables below the main tables.

With the Stable Isotope Labeling layout applied, the related tables pane opens to the Labeled Compounds per File table for L-glutamic acid.

- 3. In the related Labeled Compounds per File table, scroll to the Exchange Rate [%] column.
- 4. To view the isotopologues, click the expand icon (⊞) next to the Exchange Rate [%] column heading.

Figure 19 shows the exchange rates in the labeled (F9, F10, and F11) and unlabeled (F2, F3, and F4) samples. The exchange rates for the labeled samples are 92% for the ${}^{13}C_5H_9NO_4$ isotopologue and 7% for the ${}^{13}C_4CH_9NO_4$ isotopologue of L-glutamic acid.

The Exchange Rate [%] column contains 25 subcolumns because the analysis specified a maximum exchange of 25 atoms for any of the detected compounds. See "Analyze Labeled Compounds node" on page 17.

Color-coding:

- Irrelevant subcolumns for unprocessed elemental compositions have a gray background.
- Subcolumns for isotopologues have a pink to red background that turns darker as the exchange rate increases.

Figure 19. Exchange Rate and Study File columns in the Labeled Compounds per File table for L-glutamic acid (numbered in clockwise order, starting with 1 at the top right)



No.	Description	No.	Description
1	Unlabeled samples, F2, F3, and F4	4	Exchange rate [%] for all five carbon atoms in L-glutamic acid
2	Labeled samples, F11, F10, and F9	5	Exchange rate [%] for four out of five carbon atoms in L-glutamic acid
3	Blank sample, F1	6	Exchange rate [%] for all no carbon atoms in L-glutamic acid

View the exchange rates for the adducts of a compound

Precondition: Glutamic acid (row 2) is selected in the main Compounds table.

* To view the exchange rates for the adducts of a compound in a specific input file

- 1. In the related Labeled Compounds per File table for glutamic acid, select F11, a labeled sample.
- 2. Click Show Related Tables below the Labeled Compounds per File table.
- 3. In the related tables pane for input file F11, click the Labeled Features tab to make it the active table.

This figure shows the relative amounts (by chromatographic peak area) of the labeled adduct ions that the analysis detected for L-glutamic acid in study file F11 (a labeled sample).

Figure 20. Labeled features for L-glutamic acid in study file F11

Hide Re	lated Tabl	es																			
Compoun	ds 💡	Labeled Features	Input	Files						۳									C		
E.	Checked	lon	Charge	Molecular Weight	m/z	RT [min]	FWHM [min]	Area 🔹	Parent Area [%]		g.	Rel. Exchange [%]	Exc	har	ige	Ra	te [%	6]	1	M	Study File ID
1 👳		[M+H]+1	1	147.05316	148.06043	1.289	0.028	1665112961	83.556	1	٦.	98	0	0	0	0	7 9	2	Γ.	2	F11
2 ⊹⊐		[M+H-H2O]+1	1	147.05316	130.04987	1.288	0.028	322121115	16.164	•	4.9	98	0	0	0	0	7 9	2		9	F11
3 ⊹⊐		[M+H-NH3]+1	1	147.05316	131.03389	1.287	0.026	2957568	0.148	5	7	96	0	0	0	0	21 7	9	5	1	F11
4 ⇔		[2M+H]+1	1	147.05316	295.11359	1.289	0.027	2621939	0.132		5.0	100	0	0	1	0	0 9	9	2	8	F11

Review the labeling status

The Labeling Status column in the Compounds table and the Status column in the Labeled Compounds per File table provide information about the quality of the analysis.

- (
 Red—Indicates a contaminating mass in an unlabeled sample.
- (
 Blue—Indicates an irregular exchange rate for a labeled sample.
- (D) Orange—Indicates a low fit between the measured and fitted isotope patterns.
- (\Box) Gray—Indicates the absence of isotopologues for the detected compound.

A contaminating mass in an unlabeled sample is more problematic than an irregular exchange rate for a labeled sample.

To investigate a contaminating mass in an unlabeled sample

- In the Compounds table for the example result file, sort the compounds in descending order by Area (Max.). If you fixed L-glutamic acid to the top of the table, sorting the compounds releases it.
- 2. In row 8, click the pin icon to the left of NP-008095 to fix this compound to the top of the table.
- 3. Click the expand icon, ±, for the Labeling Status column.

Because you grouped the samples by sample type (Figure 9), the samples are also grouped by sample type in the Labeling Status column.

The red status for NP-008095 in the unlabeled samples indicates the presence of a contaminating mass in these samples (F2, F3, and F4).

Figure 21. Cuauhtemone (row 7) in the Compounds table (with the Name and Labeling Status columns frozen at the left)



- 4. In the related Labeled Compounds per File table for cuauhtemone, do the following:
 - Right-click the table and choose **Enable Column Fixing**. Then, click the pin icon for the Exchange Rate [%] column.
 - Click the expand icon, ±, for the Exchange Rate [%] column.

This figure shows the exchange rate for NP-008095 in its related Labeled Compounds per File table. The Exchange Rate [%] column shows that the contaminating mass is possibly a compound with a mass of M+4. **Figure 22**. Labeled Compounds per File table for cuauhtemone

/	Z Job Queue × Stable Isotope Labeling ×																											
	C	ompo	ounds	2	Input F	iles	Study I	Information Statistical Methods Metabolika Pathways																				
	Ē	1	Name				ц.	Labeli	ng Statu	JS																		
	8	џ	NP-008	3095																								
4																												
0) Hide Related Tables																											
1	Struc	ture l	Proposa	ls	Compou	nds per	File	Predict	ed Cor	npositi	ons	Labelec	I Comp	ounds	per File	Me	tabolika	a Result	ts m	zCloud	Results	Che	emSpid	er Resu	lts	Metabo	lika Pat	hways
	Exchange Rate [%]																									⊡ ‡		
	Ē	2		-	0	~		10	10	~	~		2	Ξ	2	2	14	5	9	2	22	6	0	5	5	8	54	52
	1	-12	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	0										
	2	4	1	0	0	0	97	0	0	0	1	0	0	0	0	0	0	0										
	3	- Þ	1	0	0	0	97	0	0	0	1	0	0	0	0	0	0	0										
	4	4	1	0	0	0	97	1	0	0	2	0	0	0	0	0	0	0										
	5	-	1	0	0	0	98	0	0	0	1	0	0	0	0	0	0	0										
	6	-	1	0	0	0	94	4	0	0	1	0	0	0	0	0	0	0										
	7	-12	1	0	0	1	97	0	0	0	1	0	0	0	0	0	0	0										
			-																									

 The false exchange rate of four carbon-13 atoms for an unlabeled compound was probably caused by a contaminating mass of M + 4.

When you apply the Stable Isotope Labeling layout, the Trend Chart view opens as a hidden view below the Isotopologues Distribution Chart view.

Use the Trend Chart view to compare the relative exchange rate [%] for each compound by input file, sample group, or study variable (for example, the time points in a metabolic flux study). When you select a single compound in the Compounds table, you can view its distribution as a box-and-whiskers plot or as a trend line plot. When you select multiple compounds in the Compounds table, the application automatically displays the distribution for each compound as a trend line plot.

Note The example data set does not include metabolic flux samples.

Tip Apply the Stable Isotope Labeling layout if you have not already applied it or if you closed some of the views. To apply the Stable Isotope Labeling layout, choose **Window > Apply Layout > Stable Isotope Labeling** from the menu bar.

- ✤ To view a box-and-whiskers plot for L-glutamic acid
- 1. To sort the main Compounds table in descending order by area, click the Area (Max.) column heading. For the example data set, L-glutamic acid sorts to the top of the table.
- 2. In the Compounds table, select L-Glutamic Acid (row 2).
- 3. In the set of tabbed views to the right of the result table, click the **Trend Chart** tab. By default, the Trend Chart view displays a box-and-whiskers plot for the relative exchange rate per group (labeled and sample).
- 4. Right-click the chart and choose **Show Legend**.

The legend appears below the chart.

5. To display a tooltip with descriptive statistics, point to a box or a whisker.

~	Show Position Tooltips
	Zoom Out
	Undo All Zoom/Pan
	Сору
	Export •
	Show Standard Errors
~	Show Legend
	Show Full Filename
_	

View a trend chart for a single compound or a set of trend lines for multiple compounds



This figure shows the box-and-whiskers plot for L-glutamic acid with the samples grouped by sample type.

View the distribution of the isotopologues for each compound Use the Isotopologues Distribution Chart view to visualize the distribution of the isotopologues for a compound.

* To view the distribution of the isotopologues for a compound

1. Apply the Stable Isotope Labeling layout to the result file (see "Apply the Stable Isotope Labeling layout" on page 22).

The Isotopologues Distribution Chart view opens to the right of the result tables.

2. In the Compounds table, select a compound of interest.

Figure 23 shows the distribution for L-glutamic acid with no sample grouping.



Figure 23. Isotopologues Distribution Chart view showing L-glutamic acid with no sample grouping

To group the samples by Sample Type, under Group By, select the Sample Type check box.
 Figure 24 shows the isotopologue distribution for L-glutamic acid with the samples grouped by sample type.
 Figure 24. Isotopologues Distribution Chart view showing L-glutamic acid with samples grouped by sample type



View the Metabolika pathways for a compound

The Map to Metabolika Pathways node (in the selected processing workflow) returns a set of mapped pathways for each detected compound.

* To view the Metabolika pathways that include a selected compound

1. Apply the Stable Isotope Labeling layout to the result file (see "Apply the Stable Isotope Labeling layout" on page 22).

The Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views open as tabbed views on the bottom right of the page. The related tables pane opens below the main tables pane.

- 2. Close some of the views as follows:
 - In the lower right quadrant, close the Isotopologues Distribution Chart view and the Trend Chart view by clicking their **Close** icons, ≤. Leave the Metabolika Pathways view open.
 - In the upper portion of the window, close the Chromatograms and Mass Spectrum views by clicking their **Close** icons, **⊠**.
- 3. Right-click the Compounds table and choose Collapse All Column Headers.
- 4. Sort the Compounds table by the Area (Max.) column in descending order.
- 5. In the Compounds table, select **row 2** (L-glutamic acid) in the example result file.
- 6. To view a Metabolika pathway that includes L-glutamic acid, do the following:
 - a. In the related tables pane for L-glutamic acid, click the Metabolika Pathways tab to make it the active table.
 - b. For this tutorial, scroll down to **row 93**—the **L-glutamate degradation IX (via 4-aminobutanoate)** pathway and select it.

This figure shows the selection of row 93 in the Metabolika Pathways table for L-glutamic acid. The mapped pathway appears in the Metabolika Pathways view at the right of the result tables. The Stable Isotope Labeling layout automatically selects the Rel. Exchange [%] column as the overlay data source with an overlay cell size of 10 pixels.

The mapped pathway appears in the Metabolika Pathways view at the right of the result tables. The Stable Isotope Labeling layout automatically selects the Rel. Exchange [%] column as the overlay data source with an overlay cell size of 10 pixels.

			Ove Sou	rlay Data rce	Overlay Cell Size	
Z Job Queue × Stable Isotope Labeling ×						- ×
🗊 Compounds 😴 Input Files Study Information Statistical Methods M	etabolika Pathways			Metabolika Pathways		≁ ‡ ×
🗭 Checked Tags 🖲 Name Formula Annot. Sour	rce 🕐 Annot. ΔMass [ppm] Calc. MW m/z RT	[min] Area (Max.) 🛁	Overlay Data Source: Rel. E	xchange [%] Y Overlay Cell Size: 1	0 🌩
2 A L-Glutamic acid C5 H9 N O4	-1.65	5 147.05291 148.06017	1.287 2076701132	Pathway: L-glutamate degr	radation IX (via 4-aminobutanoate) (#Identi	fied pathway compounds: 2)
4			•			^ (()
Hide Related Tables						-
Structure Proposals Compounds per File	Predicted Composition	s Labeled	Compounds per File	HO	-	
Metabolika Results mzCloud Results	ChemSpider Resu	lts Me	tabolika Pathways		≜	
😤 Checked Tags 💽 Pathway Name		Metabolika Compound Ids	Metabolika Compound Nar		4.1.1.15	
88 🗢 🔲 OOOOO Superpathway of plastoquinol biosynthesis		102	L-Glutamate	H _N N OH		
89 🖘 🔲 OOOOO L-tyrosine degradation IV (to 4-methylphenol)		102	L-Glutamate	0		T_aun lun
90 🖘 🔲 OOOOO Superpathway of dTDP-glucose-derived antibioti	ic building blocks biosynthe	e 102	L-Glutamate	L-Glutamate	H OOO	
91 🖘 🔲 OOOOO Superpathway of L-phenylalanine and L-tyrosine	biosynthesis	102	L-Glutamate	L-Olutamate		
92 🗢 🔲 OOOOO Superpathway of sulfolactate degradation		102	L-Glutamate			
93 🗢 🚺 OOOOO L-glutamate degradation IX (via 4-aminobutanoa	ste)	102	L-Glutamate			
94 🗢 🔲 OOOOO Superpathway of GDP-mannose-derived O-antig	en building blocks biosynth	102	L-Glutamate			
95 🖘 🔲 00000 Superpathway of rosmarinic acid biosynthesis		102	L-Glutamate			
1			*			
Show Related Tables						
0				×	,	
	Sele path	ected Metab way	olika			

c. To view the Metabolika Pathways view below the result tables instead of to their right, drag the view by its title bar until it aligns with the alignment tool's down arrow at the bottom of the page. Fi

igure 2	5. Dragging	the Metabolika	Pathways view	below the result tables
---------	-------------	----------------	---------------	-------------------------



d. Release the mouse button.

The structure for the compound that you selected in the Compounds table is blue, the structures for other detected compounds are red, and the structures for undetected compounds in the pathway are black.

- 7. To enlarge the overlaid data, increase the value in the Overlay Cell Size box (Range: 6 to 30 pixels in width).
- 8. To view the file name for a specific value, point to the value.

This figure shows the selected Metabolika pathway with an overlay of the relative exchange [%] data for the selected compound—L-glutamate. The overlay cell size is 20 pixels. A Caution symbol next to a compound indicates that the analysis found multiple matches.



9. To view information about the matching compounds for a structure with multiple matches, point to the Caution symbol.



- 10. To keep only the appropriate explanation for the structure, mark the incorrect explanation as a background compound as follows:
 - a. In the second pane of related tables, open the related Compounds table for the selected Metabolika pathway.
 - b. Fix the Name column to the leftmost column of the table. See "Common layout modifications" on page 21.
 - c. Open the Field Chooser dialog box for the related Compounds table and select the check box for the Background column.

The Background column appears in the related Compounds table.

d. To mark gamma-aminobutyric acid as a background compound, select its check box in the Background column.

🔁 Job Queue 🗙 📓 Stable Isotope Labeli	ng ×						Ŧ×
🗊 Compounds 😵 Input Files Study In	formation Statistical Methods M	letabolika Pathways					
🖆 Name 🕂 Background	[‡] Checked [‡] Tags	nula 🕂 Annot.	Sou 🛨 😑 Anno	t. ΔMass [ppm	😑 Calc. M\	N ⊕ m/z ↔	
2 4 L-Glutamic acid	C5 H	19 N O4		-1.6	55 147.05	291 148.06017	-
						Þ	
Hide Related Tables							
Structure Proposals	Compounds per File	Predicted Compo	sitions	Labe	eled Compo	unds per File	
Metabolika Results	mzCloud Results	ChemSpider	Results		Metabolik	a Pathways	
🖆 Checked Tags 🛨 Pathway N	lame		Metabolika Com	pound Ids Me	etabolika Co	mpound Names	•
93 II OOOO L-glutama	te degradation IX (via 4-aminobutano	ate)	102	L-(Glutamate		-
•	11					Þ	
Hide Related Tables							
Compounds 🌱 Metabolika Results							
🖆 Name 🕂	Metabolika Compound Names 4	Background 4	Calc. MW 🛛 🕂	m/z ⊹⊨ R	(T [min] 🗇	Area (Max.) 🔹	-12
1 😔 L-Glutamic acid	L-Glutamate		147.05291	148.06017	1.287	207670113	32
2 → L(+)-2-Aminobutyric acid	4-Aminobutanoate		103.06322	104.07050	1.398	1067182	24
3 gamma-Aminobutyric acid	4-Aminobutanoate		103.06318	104.07050	1.295	202381	10
	I						
Namo saluma fe	error at the left of	Charleba	v to poloot :	the			
Name column if							

In the Metabolika pathways view, the Caution symbol below the structure disappears, but the structure remains red.



Export the analysis results

Use the Result

Filters view to

compounds of interest

select

To create a report for your records, filter the Compounds table to display only the compounds of interest, and then export the results using the appropriate format.

Follow these procedures to filter the Compounds table and export the results:

- 1. Use the Result Filters view to select compounds of interest
- 2. Select columns that you want to export
- 3. Export the results to a spreadsheet

The analysis detected a few thousand compounds, including over one thousand compounds that it marked as background compounds or compounds without a formula. To reduce the number of compounds to export, filter the table or select the check boxes for the compounds of interest.

Note Pointing to the Compounds tab or the scroll bar for the Compounds table displays a tooltip with the number of visible compounds and the total number of compounds that the analysis detected.

	Compo	ounds 💎	Compounds per File	Features per File	Labeled (Compounds p	er File	Labeled Features
É	Þ	Tag: Compour	nds grouped by molec		Formula			
1	-12	 1960 of 3	3109 items shown (114	9 filtered out)			C10 H1	17 N3 O6 S
2	-	00000	L-Glutamic	acid			C5 H9	N 04

Do the following in the order listed:

- 1. Filter the Compounds table by the selected items
- 2. Filter the Compounds table by the relative exchange rate
- 3. Filter the Compounds table by the best mzCloud match

To filter the Compounds table by the selected items

- 1. If the Compounds table is not the active table, click its tab to make it active.
- 2. Manually select the check boxes for the compounds of interest.
- 3. From the menu bar, choose View > Result Filters.

Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the Compounds table is currently filtered by two properties—Background and Formula.

4. Click Add Property and select Checked.

This figure shows the filter set.

2 Result Filters				\times
ON Compounds ON Compounds per File ON Features per File ON Labeled Compounds per File ON Labeled Features ON Metabolika Results ON ChemSpider Results ON Input Files ON Study Information ON Statistical Methods ON Metabolika Pathways	Compounds AND Add group Formula is not blank Remove Background is false Remove - Checked is true Remove Add property			
Show all tables	Load Save Save As Clear All C	Clear	Apply F	ilters

5. Click Apply Filters.

The Compounds table displays only the selected compounds.

6. To undo the Checked filter, click **Remove** to its right. Then, click **Apply Filters** again.

Now that you have removed the Checked filter, the Compounds table contains the original set of compounds.

Go to the next topic to "Filter the Compounds table by the relative exchange rate."

Filter the Compounds table by the selected items

Filter the Compounds table by the relative exchange rate

* To filter the Compounds table by the relative exchange rate of each compound

- 1. Click the **Compounds** tab for the main Compounds table to make it the active table.
- 2. From the application menu bar, choose **View > Result Filters**.

The Result Filters view opens as a floating window. Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the filter for the Compounds table already includes a filter for background compounds and a filter for compounds without a formula.

This figure shows the default filters for the example result file.

② Result Filters	— 🗆 X
ON Compounds ON Compounds per File ON Features per File ON Labeled Compounds per File ON Labeled Features ON Labeled Features ON Metabolika Results ON ChemSpider Results ON Input Files ON Study Information ON Statistical Methods ON Metabolika Pathways	Compounds AND Add group Formula is not blank Remove Background is false Remove Add property
Show all tables	Save Save As Clear All Clear Apply Filters

- 3. On the right side of the Result Filters view, set up filters for the relative exchange rate as follows:
 - a. Click Add Property, and then select Rel. Exchange [%] from the list.
 - b. In the pink relation list, select Is Greater Than or Equal To.
 - c. In the value box next to the relation list, type 98.
 - d. In the pink condition list, select In File.
 - e. In the Green sample list, select one of the labeled input files.
 - f. Repeat steps step 3a through step 3e to add a filter for all three labeled input files.

This figure shows the filter set.

② Result Filters	- 🗆 X
ON Compounds ON Compounds per File ON Features per File ON Labeled Compounds per File ON Labeled Features ON Labeled Features ON Metabolika Results ON ChemSpider Results ON Study Information ON Statistical Methods ON Metabolika Pathways	Compounds AND Add group Formula is not blank Remove Background is false Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_01.raw (F9) Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_02.raw (F10) Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_03.raw (F11) Remove Add property
Show all tables	Load Save Save As Clear All Clear Apply Filters

4. Click Apply Filters.

The applied filter set reduces the number of visible rows in the Compounds table to 236.

Com	pounds	8	Compounds per File	Features per File	Labeled Compounds
Ē	Tags	े Comp	ounds grouped by mole	ecular weight and ret	ention time
1 🕀	00	236 o	of 3109 items shown (287	73 filtered out)	

Leave Result Filters view open and go to the next topic to filter the remaining visible compounds by their best mzCloud match scores.

Filter the Compounds table by the best mzCloud match Precondition: The Compounds table in the example result file is filtered by the exchange rate as described in "Filter the Compounds table by the relative exchange rate" on page 34.

* To filter the Compounds table by the best mzCloud match

- 1. Open the Result Filters view if it is not open.
- 2. Click Add Property below the last Rel Exchange [%] filter.
- 3. In the dropdown property list, select mzCloud Best Match.
- 4. In the pink dropdown conditions list to the right, select Is Greater Than or Equal To.
- 5. In the value box, type **99**.

② Result Filters	— D X
ON Compounds ON Compounds per File ON Features per File ON Labeled Compounds per File ON Labeled Features ON Labeled Features ON Metabolika Results ON ChemSpider Results ON Input Files ON Study Information ON Statistical Methods ON Metabolika Pathways	Compounds AND Add group Formula is not blank Remove Background is false Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_01.raw (F9) Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_02.raw (F10) Remove Rel. Exchange [%] is greater than or equal to 98.00 in file Ecoli_13C_03.raw (F11) Remove Macloud Best Match is greater than or equal to 99 Remove Add property
Show all tables	Load Save Save As Clear All Clear Apply Filters

6. Click Apply Filters.

The Compounds table is now reduced to three compounds.

		Compo	ounds 💎	Compoun	ds per File	Features per File
I		F	Tags !	Checked	Name	
I	1	-12	00000		L-Glutamic	acid
I	2	-12	00000		DL-Glutam	ine
	3	-12	00000		Y-L-Glutarr	ıyl-L-glutamic acid

7. Close the Result Filters view by clicking the **Close** icon, \times , in the upper-right corner.

Go to the next topic to "Export the results to a spreadsheet."

Select columns that you want to export For the stable isotope labeling analysis, the Compounds table contains up to 37 visible table columns. With the Stable Isotope Labeling layout applied, the Compounds table contains 19 visible table columns. See the Field Chooser dialog box in the following topic: "Apply the Stable Isotope Labeling layout" on page 22.

* To select the table columns that you want to export to a spreadsheet file

- 1. Click the Field Chooser icon, *F*, for the Compounds table.
- 2. In the Field Chooser dialog box, clear the check boxes for the columns that you do not want to export to a spreadsheet file. For this tutorial, clear all the check boxes except the following five columns:

Name, Area (Max), Calc. MW, Formula, and RT

3. Close the Field Chooser dialog box.

Export the results to a spreadsheet

Preconditions: The Compounds table is filtered by a relative exchange rate of greater than or equal to 98% and an mzCloud Best Match of greater than or equal to 99% and contains only two compounds and only six columns.

Note If you have not already filtered the Compounds table and reduced the number of visible table columns, see these topics:

- Filter the Compounds table by the relative exchange rate
- Filter the Compounds table by the best mzCloud match
- Select columns that you want to export

To create a report by exporting the results to a spreadsheet

- 1. Sort the Compounds table in descending order by the Area (Max) column.
- 2. To export the filtered and sorted results, do the following:
 - a. Right-click the Compounds table and choose **Export > As Excel**.

C, Job	Qu	ueue × / 📓 Stable Isotope	abeling ×								
Com	npo	unds 😵 Compounds per	File Features p	er File	abeled Com	pounds p	er File	Labele	ed Features	mzCloud Results	
F		Name	Formula		Calc. MW	RT [min]	Area (N	/lax.) 🔻			
1 👳		L-Glutamic acid	C5 H9 N O4		147.05291	1.287	2076	701132			
2 ⊹⊐		DL-Glutamine	C5 H10 N2 O3		146.06891	1.250	623	371343			
3 🕀		Y-L-Glutamyl-L-glutamic acid	C10 H16 N2 O7		276.09554	1.694	30	045026			
		Copy With Headers Copy Copy Structure Clear Selection Cell Selection Mode Enable Column Fixin Collapse All Column I Expand All Column I Check Selected	g Headers Headers	Ctrl+C	 						
		Uncheck Selected			•						
		Uncheck All			*						
		Kemove All Checkm	arks in All Tables		As	Plain Text	t				
		Add Tag			As	Excel					
		Remove lag			As	mzVault I	Library	13			
		Set lags	II Tablas		Ac	ld Compo	und to I	Existing	mzVault Libra	ary	
		Remove Air lags in A	ai lables		As	Xcalibur	Inclusio	n/Exclusi	on List	-	
		Edit Compound Ann	otation		As	TraceFind	ler List	, energia	er erstin		
		Clear Compound An	notation		10	Mageliet					
		Apply FISh Scoring			As	iviass List	 d Comn	ounds to	- Evicting Ma	acc List	
		Export			► AC	iu selecte	u comp	ounds to	s chisting wa	ISS LIST.	

The Export to Excel dialog box opens.

ath:			
Items and	d related tables to be exported		Options
Level 1: Level 2: Level 3:	Compounds	 	 All items Selected in this table Selected in this and all sub-tables Open file after export

b. In the Path box, change the file name and the location of the spreadsheet file as appropriate by clicking the browse icon, selecting the storage folder, naming the file, and clicking **Save**.

The dialog box remembers the last folder location you selected. The default file name is the table name.

- c. In the Items and Related Tables to be Exported area, do the following:
 - Do not change the Level 1 selection of the Compounds table.
 - In the Level 2 list, select Labeled Compounds per File.
- d. In Options area, select the All Items option and the Open File After Export check box.

② Export	to Excel		×
Path: C:\Users\F Items and Level 1: Level 2: Level 3:	ublic\Documents\Stable Isotop I related tables to be exported Compounds Labeled Compounds per File	v v v	Uning.xlsx Options All items Selected in this table Selected in this and all sub-tables Open file after export
			Export Cancel

- e. Click Export.
- f. At the status prompt, click OK.



The Excel spreadsheet opens (Figure 26).

This spreadsheet shows the exported Compounds table.

Figure 26. Excel spreadsheet with three compounds and the labeled compounds per file for each of the three compounds

	Aut	oSav	e 💽 🖁	ら 、	- B	÷	Stable Is	otope La	abeling.xls>	: - Excel	ې	o c
	File	ł	Home Insert	Page L	ayout I	Formul	as Data	Review	v View	Add-ins	Help	ACROB,
	A1		• E 2	< 🗸	$f_{\mathcal{K}}$	Name	2					
	1 2			А			В		С	D		E
		1	Name				Formula	C	alc. MW	RT [min]	Area (Max.)
	+	2	L-Glutamic ac	id			C5 H9 N O4	L :	147.0529	1.287	207	6701132
	+	11	DL-Glutamine				C5 H10 N2	O3	146.0689	1.25	6237	1342.88
	+	20	Y-L-Glutamyl-	L-glutam	ic acid		C10 H16 N2	207	276.0955	1.694	3045	026.195
		28										
Expand	-	÷	Compo	unds	(+)							4
icon												

3. To view the Compounds per File table for a compound, click the expand icon, ⊞, to the left of the compound. The Labeled Compounds per File table for the compound expands below the compound.

AutoSave 💽 🛱 🏷 Y 🖓 Y Stable Isotope Labeling.xlsx - Excel 🖉 Search													
File		Home	Insert	Page Layou	t Formulas	Data Revie	w View Ado	l-ins Help					
AN3	AN3 💌 i 🔀 🗸 🏂 Study File ID												
1 2			А		В	С	D	E	F	G	н	1	
	1	Name			Formula	Calc. MW	RT [min]	Area (Max.)					
-	2	L-Glutam	ic acid		C5 H9 N O4	147.05291	1.287	2076701132					
· ·	3				Checked	Tags	Molecular Weight	RT [min]	FWHM [m	Max. # MI	# Adducts	Area	
· ·	4				FALSE		147.05291	1.286	0.025	4	4	2.08E+09	
· ·	5				FALSE		147.05291	1.288	0.028	3	4	2.05E+09	
· ·	6				FALSE		147.05291	1.287	0.029	3	4	2.04E+09	
· ·	7				FALSE		147.05291	1.289	0.028	5	4	1.99E+09	
· ·	8				FALSE		147.05291	1.288	0.026	5	4	1.96E+09	
· ·	9				FALSE		147.05291	1.286	0.026	4	4	1.93E+09	
Ŀ	10				FALSE		147.05291	1.378	0.025	2	3	176615.1	
+	11	DL-Glutar	mine		C5 H10 N2 O3	146.06891	1.25	62371342.88					
-	Compounds												

Trademarks

Compound Discoverer, LTQ Orbitrap XL, LTQ Orbitrap Velos, Q Exactive Plus, mzCloud, Orbitrap ID-X, Orbitrap Elite, Orbitrap Velos Pro, and Q Exactive are trademarks; and Exactive, Orbitrap, Orbitrap Fusion, and Xcalibur are registered trademarks of Thermo Fisher Scientific Inc. in the United States.

ChemSpider is a trademark of ChemZoo, Inc.

Microsoft and Excel are registered trademarks of Microsoft Corporation in the United States and other countries.

All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.