

Thermo

# EASY-nLC Series

## User Guide for the Xcalibur Data System

Xcalibur 2.1 or later

60053-97230 Revision C November 2013



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**For Research Use Only. Not for use in diagnostic procedures.**

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# Preface

This guide describes how to configure the Thermo EASY-nLC™ virtual instrument (VI) control software from the Thermo Foundation™ platform, set up the liquid chromatography (LC) parameters for LC/MS instrument methods in the Instrument Setup window of the Thermo Xcalibur™ data system, and set up the sample position settings and injection volumes for an injection sequence from the Sequence Setup view in the Xcalibur data system.

**IMPORTANT** To establish communication with the EASY-nLC instrument and to avoid the premature termination of data acquisition during sequence runs, set up the data system computer as described in “[Data System Computer Settings](#)” on [page viii](#).

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- [Related Documentation](#)
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### ❖ To suggest changes to the documentation or to the Help

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.



## Related Documentation

Thermo Fisher Scientific provides these manuals as PDF files on the data system computer and on the USB flash drive that ships with the EASY-nLC instrument:

- *EASY-nLC Series Preinstallation Requirements Guide*
- *EASY-nLC Series Getting Started Guide* (using the stand-alone, touch-screen application)
- *EASY-nLC Series Troubleshooting and Maintenance Guide*
- *EASY-nLC Series User Guide for the Xcalibur Data System*

The data system also provides Help for the EASY-nLC virtual instrument control software.

### ❖ To access the EASY-nLC manuals on the data system computer

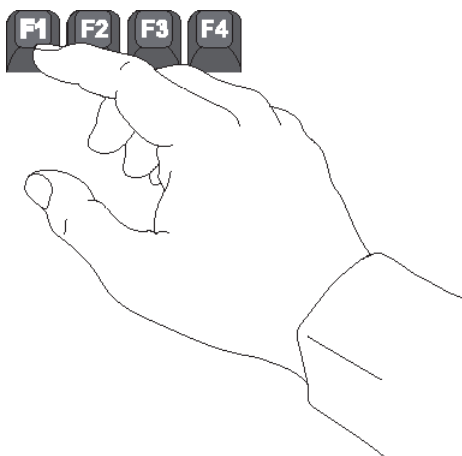
Choose **Start > Programs** (or **All Programs**) > **Thermo Instruments > Manuals > LC Devices > Thermo**.

### ❖ To open the Help from the EASY-nLC view of the Instrument Setup window

- To open the Welcome page of the Thermo EASY-nLC Help, choose **Help > Thermo EASY-nLC Help** from the menu bar.
- To open the Help topic for the current page of the Thermo EASY-nLC view, do one of the following:
  - Choose **Help > Help On Current Item F1** from the menu bar.
  - Press the F1 key on the computer keyboard.

### ❖ To open the Help from the Thermo EASY-nLC Configuration dialog box of the Foundation Instrument Configuration window

Press the F1 key on the computer keyboard.



## System Requirements

Table 1 lists the data system computer hardware and software requirements for the EASY-nLC device driver. This device driver is provided on the LC Devices DVD and is designed for use with the Thermo Xcalibur data system or an equivalent Thermo Scientific mass spectrometry application.

**IMPORTANT** Before you install the device driver provided on the LC Devices DVD, ensure that the data system computer has a compatible version of the Foundation platform as noted in the *Thermo LC Devices x.x.x Release Notes*, where *x.x.x* is the version you want to install. You can find the Release Notes document, which includes the system requirements, on the LC Devices DVD.

**Table 1.** Data system computer hardware and software requirements

Hardware	Minimum requirements	
Data system computer	<ul style="list-style-type: none"> <li>• 2 GHz processor with 1 GB RAM</li> <li>• DVD-ROM drive</li> <li>• 80 GB hard drive capacity</li> <li>• Display resolution 1280 × 1024 resolution</li> <li>• 2 Network cards</li> <li>• NTFS format</li> </ul>	
Software	Requirements	
Operating system	Microsoft™ Windows™ operating system: <ul style="list-style-type: none"> <li>• Windows 7 Professional (32-bit and 64-bit<sup>a</sup>)</li> </ul> –or– <ul style="list-style-type: none"> <li>• Windows XP Workstation SP3</li> </ul>	
Third-party software	Adobe™ Reader™ 9.0 or later	
Thermo Scientific software	<b>LC Devices</b>	<b>Foundation platform</b>
	<b>EASY-nLC device driver</b>	
	2.8.0 or later	1.0.2 SP2 or later
	2.7.0	1.0.2 SP2 or 2.0.0 SP1

<sup>a</sup> LC Devices 2.8.0 and later are compatible with Windows 7, 32-bit and 64-bit

## Data System Computer Settings

Table 2 lists the computer settings that are required to avoid the premature termination of data acquisition during sequence runs. It also provides the virus scanner setting that is required to establish communication with the EASY-nLC instrument.

**IMPORTANT** After you install the Thermo EASY-nLC device driver on a Windows 7 computer, exclude the Thermo EASY-nLC software from the Prevent IRC Communication list for the computer's virus scanner (see [“Excluding the EASY-nLC from the VirusScan Enterprise”](#) on page 5).

If you do not exclude the Thermo EASY-nLC software from the Prevent IRC Communication list, the data system computer cannot establish communication with the EASY-nLC instrument.

**Table 2.** Data system computer settings

Category	Settings
Power saver	<ul style="list-style-type: none"> <li>• Screen saver: None</li> <li>• Hibernate: Not enabled or Never</li> <li>• Turn off hard disk: Never</li> <li>• Turn off monitor: Never</li> </ul>
Windows updates	If the data system computer is connected to the Internet, turn off the automatic Windows updates.
Virus scanner	For the Windows 7 operating system, exclude the Thermo EASY-nLC software from the virus scanner.



## Cautions and Special Notices

Make sure you follow the cautions and special notices presented in this guide. Cautions and special notices appear in boxes; those concerning safety or possible system damage also have corresponding caution symbols.

This guide uses the following types of cautions and special notices.



**CAUTION** Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

## Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ **To contact Technical Support for the EASY-nLC instrument**

Phone	800-532-4752
Fax	561-688-8736
Web site	<a href="http://www.proxeon.com">www.proxeon.com</a>
E-mail	(North and South America) <a href="mailto:us.techsupport.analyze@thermofisher.com">us.techsupport.analyze@thermofisher.com</a> (Other continents) <a href="mailto:eu.techsupport.cmf@thermofisher.com">eu.techsupport.cmf@thermofisher.com</a>
Knowledge base	<a href="http://www.thermokb.com">www.thermokb.com</a>

Find software updates and utilities to download at [mssupport.thermo.com](http://mssupport.thermo.com).

❖ **To order consumable and spare parts for the EASY-nLC instrument**

For the EASY-nLC 1000 instrument, go to

[www.proxeon.com/productrange/nano\\_lc\\_easy-nlc\\_1000/accessories\\_spares/index.html](http://www.proxeon.com/productrange/nano_lc_easy-nlc_1000/accessories_spares/index.html).

For the EASY-nLC II instrument, go to

[www.proxeon.com/productrange/nano\\_lc/accessories-spares/index.html](http://www.proxeon.com/productrange/nano_lc/accessories-spares/index.html).

❖ **To get local contact information for sales or service**

Go to [www.thermoscientific.com/wps/portal/ts/contactus](http://www.thermoscientific.com/wps/portal/ts/contactus).

❖ **To copy manuals from the Internet**

Go to [mssupport.thermo.com](http://mssupport.thermo.com), agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

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- Fill out a reader survey online at [www.surveymonkey.com/s/PQM6P62](http://www.surveymonkey.com/s/PQM6P62).
- Send an e-mail message to the Technical Publications Editor at [techpubs-lcms@thermofisher.com](mailto:techpubs-lcms@thermofisher.com).

# Installing the EASY-nLC Control Software

This chapter describes how to install the EASY-nLC virtual instrument (VI) control software from the LC Devices DVD. It also describes how to ensure communication between the data system computer and the EASY-nLC instrument.

## Contents

- [Checking the System Prerequisites](#)
- [Removing a Previous Version of the Instrument Control Software](#)
- [Installing the Current Version of the Instrument Control Software](#)
- [Excluding the EASY-nLC from the VirusScan Enterprise](#)

## Checking the System Prerequisites

The LC/MS system consists of the EASY-nLC instrument, a Thermo Scientific mass spectrometer, and a data system computer.

Before installing the EASY-nLC VI control software, do the following:

- Install compatible versions of the Thermo Foundation platform and the Xcalibur data system (or equivalent Thermo Scientific data system).
- Ensure that you have administrator rights to install the EASY-nLC VI control software for the Xcalibur application.
- Remove any earlier version of the EASY-nLC VI control software from the data system computer (see [“Removing a Previous Version of the Instrument Control Software”](#) on [page 2](#)).

## 1 Installing the EASY-nLC Control Software

### Removing a Previous Version of the Instrument Control Software

Before or after installing the EASY-nLC VI control software, do the following:

- Connect the EASY-nLC instrument to the data system computer through an Ethernet connection (see “[Connecting the EASY-nLC Instrument to the Data System Computer](#)” on [page 7](#)).
- Connect the EASY-nLC instrument to the Thermo Scientific MS detector with a contact closure cable.

The data system synchronizes the run timing between the LC system and the mass spectrometer through the cable. For information about connecting the contact closure cable, refer to the *EASY-nLC Series Getting Started Guide*.

- Check the laboratory local area network (LAN) port for Internet access to the remote support server. For remote diagnostics, the laboratory must have a LAN port with Internet access.

The network firewall must allow outgoing Transmission Control Protocol/Internet Protocol (TCP/IP) traffic from the EASY-nLC instrument to the remote support server at IP address 195.41.108.93, port 22.

After installing the EASY-nLC VI control software, exclude the software from the virus scanner as described in “[Excluding the EASY-nLC from the VirusScan Enterprise](#)” on [page 5](#).

## Removing a Previous Version of the Instrument Control Software

### ❖ To remove a previously installed version of the EASY-nLC Xcalibur VI

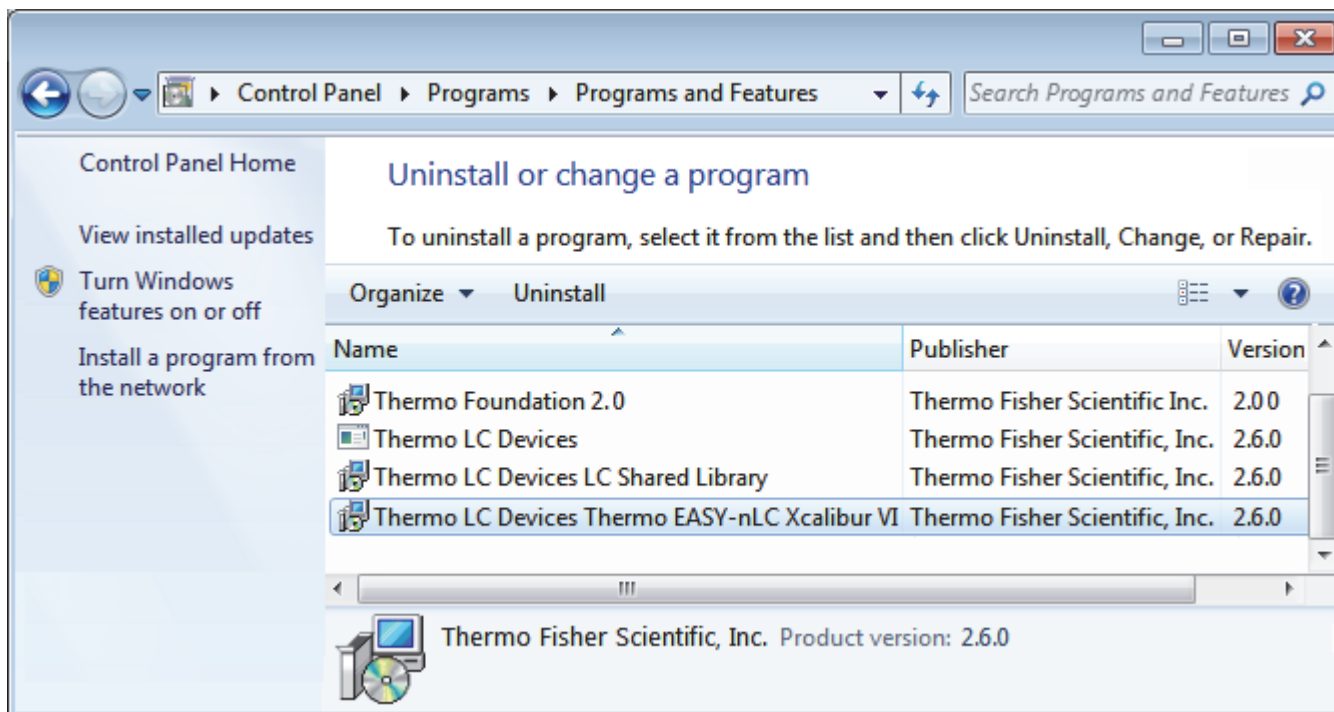
1. Depending on the Windows operating system, do one of the following:

- For Windows 7, choose **Control Panel > Uninstall a Program**.

The list of installed programs appears (see [Figure 1](#)).

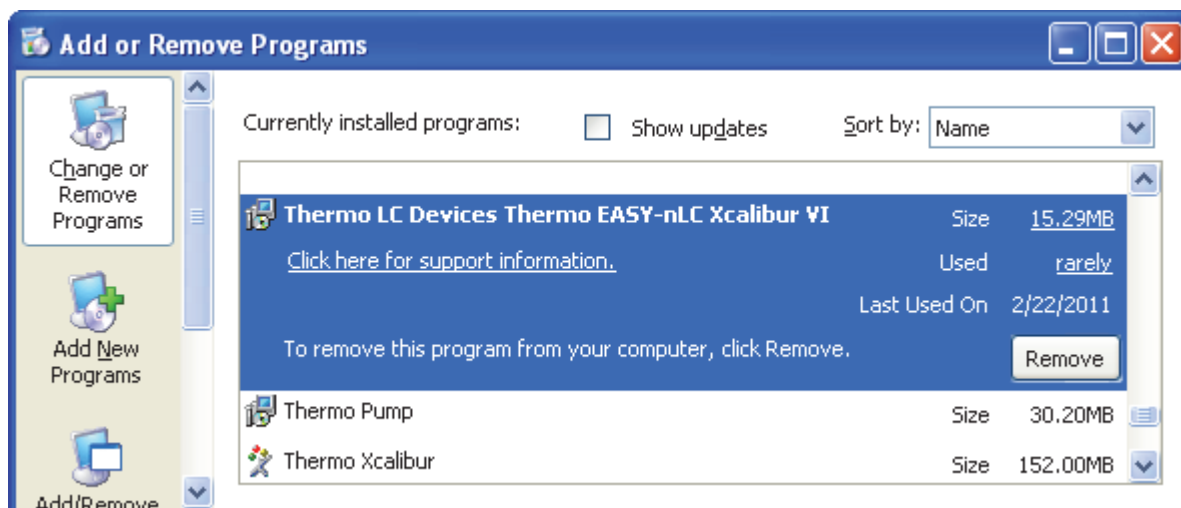
- For Windows XP, choose **Start > Settings > Control Panel > Add or Remove Programs**.

Figure 1. Uninstall window for the Windows 7 operating system



2. In the list of installed programs, locate **Thermo LC Devices Thermo EASY-nLC Xcalibur VI**.
3. Depending on the operating system, do one of the following:
  - For Windows 7, double-click **Thermo LC Devices Thermo EASY-nLC Xcalibur VI** or click **Uninstall**. Wait for the program to be removed from the list.
  - For Windows XP, click **Remove** (see Figure 2). Wait for the program to be removed from the list.

Figure 2. Add or Remove Programs window for the Windows XP operating system

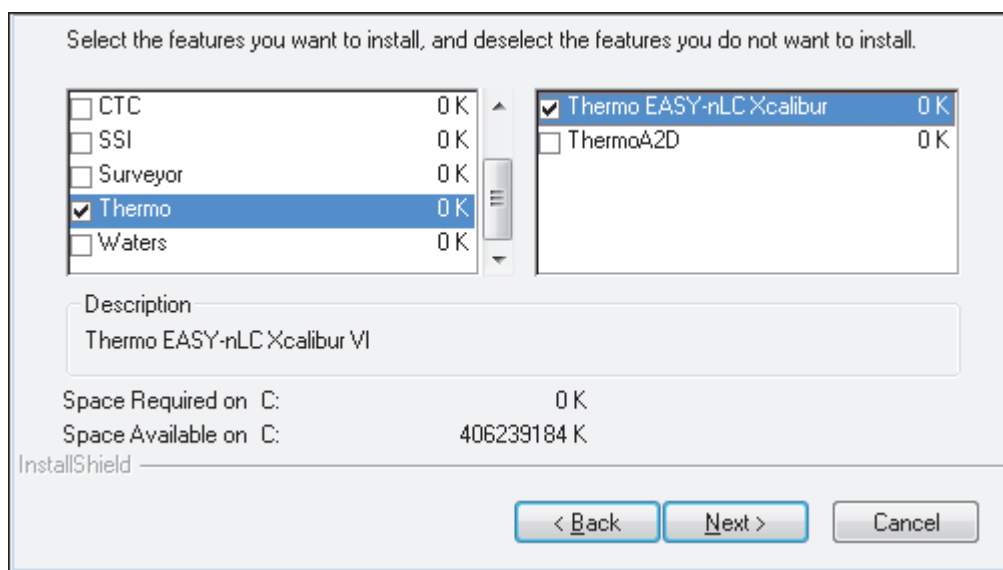


# Installing the Current Version of the Instrument Control Software

### ❖ To install the EASY-nLC Virtual Instrument software

1. Check the installed versions of the Foundation platform and the Xcalibur data system and make sure that these versions are compatible with the LC Devices version that you are installing (see “System Requirements” on page vii).
2. Close all running programs before starting the installation.
3. If applicable, remove the previously installed version of the EASY-nLC VI software (see “Removing a Previous Version of the Instrument Control Software” on page 2).
4. Load the LC Devices DVD into the data system computer, and follow the instructions in the Thermo LC Devices InstallShield Wizard.
5. On the Select Features page of the wizard, select the **Thermo** check box on the left, and then select the **Thermo EASY-nLC Xcalibur** check box on the right (see Figure 3).

**Figure 3.** Select Features page of the Thermo LC Devices InstallShield Wizard



6. When the installation finishes, restart the data system computer.


The installation is complete when you log back in.

## Excluding the EASY-nLC from the VirusScan Enterprise

The McAfee™ VirusScan™ Enterprise software blocks the Thermo EASY-nLC software when the data system computer uses the Windows 7 operating system.

**IMPORTANT** For the Windows 7 operating system, you must exclude the EASY-nLC VI software from the McAfee blocking list. For the Windows XP operating system, exclude the EASY-nLC VI software from the McAfee blocking list only if the EASY-nLC instrument is experiencing communication problems.

### ❖ To exclude the EASY-nLC software from the VirusScan Enterprise 8.7.0i software

1. Log in to the data system computer as an administrator.
2. Open the McAfee VirusScan Console application as follows:
  - a. Do one of the following:
    - On the Windows 7 taskbar, right-click the **McAfee** icon, , and choose **Manage Features > VirusScan Enterprise**.
  - or–
  - From the Windows 7 desktop, choose **Start > All Programs > McAfee > VirusScan Console**.
3. In the Task column, right-click **Access Protection** and choose **Properties** from the shortcut menu.

The Access Protection Properties dialog box appears.

4. To open the Rules dialog box, do the following:
  - a. Under Categories, choose **Anti-virus Standard Protection**.
  - b. Under Rules, select **Prevent IRC Communication**.
  - c. Click **Edit**.

The Rules Details dialog box opens.

5. Modify these settings:
  - In the Processes to Include box, delete the asterisk.

**Note** When the Processes to Include box contains only a single asterisk, the virus scanner prevents IRC communication for all processes.

- In the Processes to Exclude box, type **Thermo\***.

**Note** When the Processes to Exclude box contains the text **Thermo\***, the Access Protection service for Internet Relay Chat (IRC) communication processes ignores processes that begin with Thermo.

## 1 Installing the EASY-nLC Control Software

Excluding the EASY-nLC from the VirusScan Enterprise

6. Click **OK** to close the Rules Details dialog box.
7. Click **OK** to close the Access Protection Properties dialog box and accept the new settings.
8. Close the VirusScan Console application.



## Setting Up the Foundation Instrument Configuration

This chapter describes how to set up the instrument configuration for the EASY-nLC device driver.

Before you set up the instrument configuration, connect the EASY-nLC instrument to the data system computer, turn on the EASY-nLC instrument, and log in to the instrument as an administrator. Then, use the Thermo EASY-nLC Configuration dialog box of the Thermo Foundation Instrument Configuration window to set up and test the network connection for the EASY-nLC instrument, to specify the tray type that you plan to use, and to identify the mobile phase solvents in solvent reservoir bottles A and B.

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- [Connecting the EASY-nLC Instrument to the Data System Computer](#)
- [Adding the System Devices to the Instrument Configuration](#)
- [Setting Up the EASY-nLC Configuration](#)
- [Setting Up the Sample Layout and Start Position](#)

## Connecting the EASY-nLC Instrument to the Data System Computer

The EASY-nLC instrument and the Thermo Scientific mass spectrometer communicate with the data system computer through an Ethernet connection routed through an Ethernet switch connected to the data system computer.

These are the default IP addresses of the data system computer and the EASY-nLC instrument.

**Table 3.** Default IP addresses for the LC system

System module	IP address
Data system computer	172.16.0.101
EASY-nLC instrument	172.16.0.103

## 2 Setting Up the Foundation Instrument Configuration

Adding the System Devices to the Instrument Configuration

### ❖ To connect the EASY-nLC instrument to the data system computer

1. Connect a shielded Ethernet cable to the LAN port on the back panel of the EASY-nLC instrument.
2. Connect the other end of the Ethernet cable to an Ethernet switch.
3. Using a second shielded Ethernet cable, connect the Ethernet switch to the appropriate network card installed in the data system computer.

For more information about setting up the communication cables, refer to the *EASY-nLC Series Getting Started Guide*.

**IMPORTANT** Avoid IP conflicts with other units on the network, especially the mass spectrometer.

## Adding the System Devices to the Instrument Configuration

Your Thermo Scientific LC/MS system consists of a Thermo Scientific mass spectrometer and an EASY-nLC instrument. To control these instruments from the Xcalibur data system, you must add the installed device drivers to the instrument configuration.

**Note** The Xcalibur data system and the Foundation Instrument Configuration window cannot be open simultaneously.

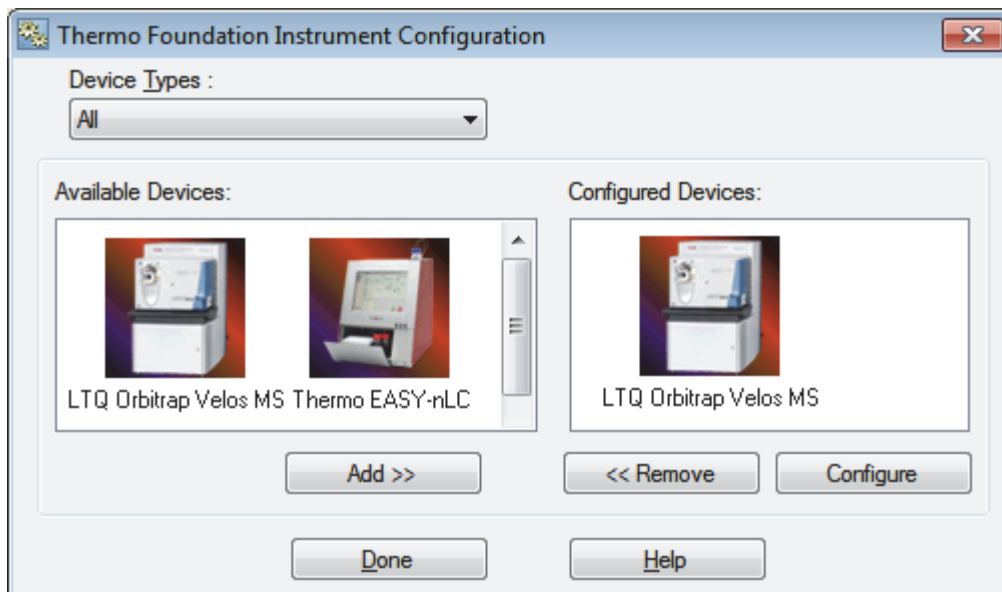
### ❖ To add devices to the instrument configuration

1. Close the Xcalibur data system if it is open.
2. From the Windows taskbar, choose **Start > Programs (or All Programs) > Thermo Foundation x.x > Instrument Configuration**, where *x.x* is the installed version of Thermo Foundation.

The Thermo Foundation Instrument Configuration window opens (see [Figure 4](#)). The installed devices are listed in the Available Devices list. The configured devices are listed in the Configured Devices list.

[Figure 4](#) shows an instrument configuration with a Thermo Scientific mass spectrometer added to the Configured Devices list.

Figure 4. Thermo Foundation Instrument Configuration window



**Tip** To access Help, press F1 or click **Help**.

3. Add the EASY-nLC instrument to the instrument configuration as follows:
  - a. Make sure that the Device Types list is set to **All** or **AS** (Autosampler).  
If the Device Type list is set to LC, the EASY-nLC VI for Xcalibur device does not appear in the Available Devices list.
  - b. In the Available Devices list, double-click the **Thermo EASY-nLC** icon.  
A copy of the Thermo EASY-nLC icon appears in the Configured Devices list.
4. Set up the configuration options for each configured device.  
For information about the EASY-nLC configuration parameters, see the next topic, [“Setting Up the EASY-nLC Configuration.”](#)
5. Click **Done** to close the Thermo Foundation Instrument Configuration window.

**Tip** You must close the Instrument Configuration window before you open the Xcalibur data system. The two applications cannot be open at the same time.

## Setting Up the EASY-nLC Configuration

Use the Thermo EASY-nLC Configuration dialog box to set up and test the communication connection to the data system computer, to specify the autosampler model and the plate type that you are using, and to identify the mobile phase solvents. Clicking the Edit button in the Autosampler Configuration area opens the Sample Position Selector dialog box where you set up the sample layout (row first or column first) and the start position for an Xcalibur injection sequence.

### ❖ To set up the EASY-nLC configuration in the Foundation platform

1. If you have not already added the EASY-nLC instrument to the Configured Devices list of the Thermo Foundation Instrument Configuration window, add it as described in [“Adding the System Devices to the Instrument Configuration”](#) on page 8.
2. In the Configured Devices list of the Thermo Foundation Instrument Configuration window, double-click the **Thermo EASY-nLC** icon.

The Thermo EASY-nLC Configuration dialog box opens (see [Figure 5](#)).

**Figure 5.** Thermo EASY-nLC Configuration dialog box

Thermo EASY-nLC Configuration

EASY-nLC Network Configuration

IP Address/Hostname:  The IP Address can be found in the About box on the EASY-nLC instrument

User name:  Run samples as this user (User must exist on the EASY-nLC)

Click 'Test Connection' to test the connection

LC device driver and instrument information:

LC device driver : LCServer 1.4.2.1

Autosampler Configuration

Autosampler:

Plate installed:  Edit...

Sample layout:  Start pos:

Solvents for LC Pump

A:

B:

3. Ensure the following:
  - The EASY-nLC instrument is connected to the data system computer by way of an Ethernet switch (see [“To connect the EASY-nLC instrument to the data system computer”](#) on page 8).
  - The EASY-nLC instrument is turned on, and you are logged in as an administrator to the instrument.

4. Set up the network configuration for the EASY-nLC instrument as follows:

- a. Type the IP address of the EASY-nLC instrument and your user name in the appropriate boxes.

You must have an account on the EASY-nLC instrument with the same user name. The default user name is “admin,” but you can change this to any existing user account on the EASY-nLC instrument.

**Tip** The EASY-nLC IP address is listed in the About box, which you can open by pressing the THERMO logo in the upper-left corner of the touch screen. The current user is listed in the lower-left corner of the touch screen.

If necessary, change the IP address on the Configuration > Network page of the touch-screen software.

- b. Click **Test Connection** to test the connection to the EASY-nLC instrument and to download the instrument configuration information to the data system computer.
5. Check the message in the status box to the right of the Test Connection button.
    - If the connection is established, the status box changes to green (see [Figure 6](#)) and the information about the LC device driver and instrument appears.
    - If a connection is not established, the status bar changes to red. Check the Ethernet cable connections, the IP address of the EASY-nLC instrument, and the IP address of the network card installed in the data system computer (see [“Connecting the EASY-nLC Instrument to the Data System Computer”](#) on page 7).
    - If an antivirus program or firewall blocks the connection, the application displays this error message:

An established connection was aborted by the software in your host machine.

Contact your local IT administrator for assistance.

For information about excluding the EASY-nLC software from the McAfee virus scanner, see [“Excluding the EASY-nLC from the VirusScan Enterprise”](#) on page 5.

6. Check the instrument information. If necessary, change the instrument information as described in [“To change the loop volume, column setup, idle mixture, or idle flow rate”](#) on page 13.

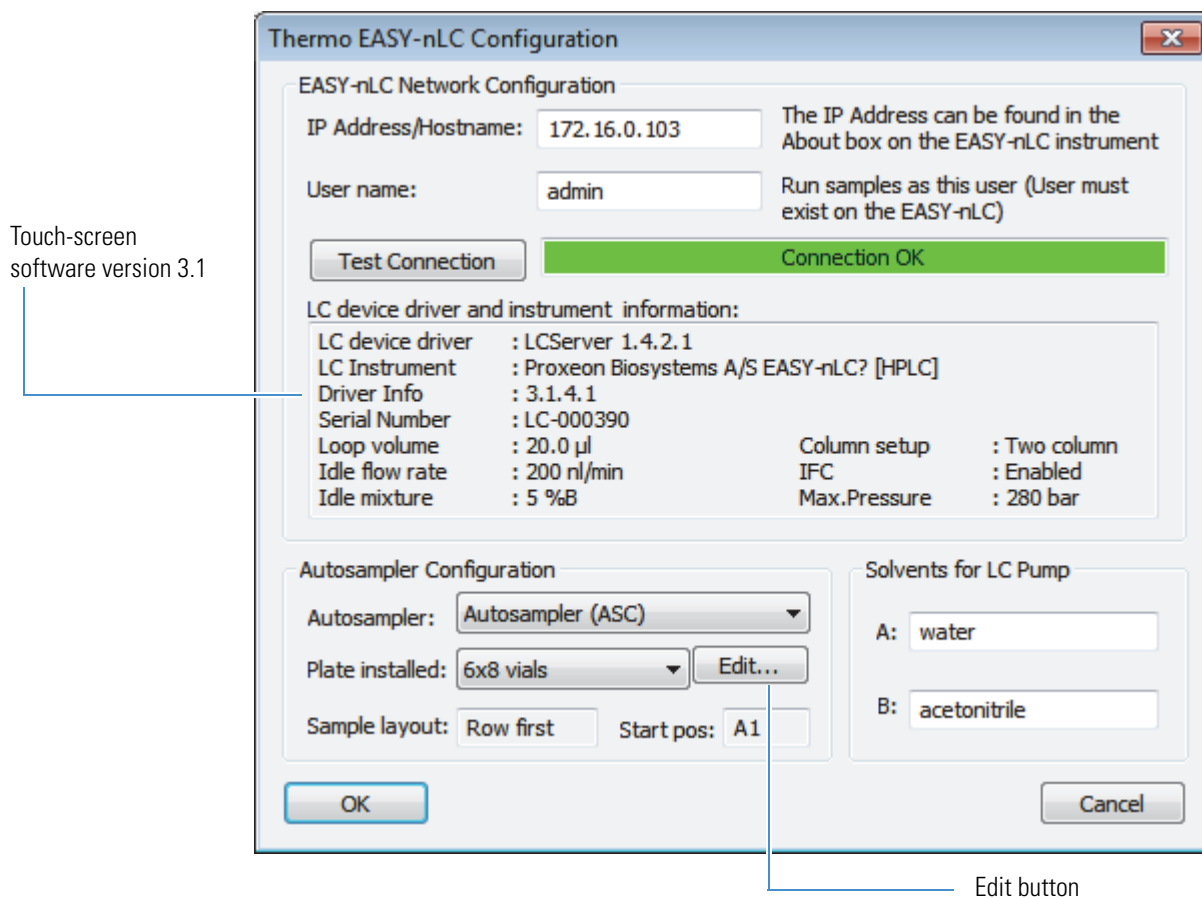
## 2 Setting Up the Foundation Instrument Configuration

Setting Up the EASY-nLC Configuration

7. Set up the autosampler configuration in the Autosampler Configuration area as follows:
  - a. Select the autosampler model (**Autosampler ASA** or **Autosampler ASC**).
  - b. Select the plate type (**96-** or **384-well plates**, or a **4 × 6 (ASA)** or **6 × 8 (ASC)** vial holder).

**Note** All plate formats for the ASC model have six extra vial positions on the side. When you set up an Xcalibur sequence, these vials are numbered 1-V1 to 1-V6.

**Figure 6.** Thermo EASY-nLC Configuration dialog box



- c. (Optional) Set up the default sample layout and start position to be used when creating a new Xcalibur sequence as described in “[Setting Up the Sample Layout and Start Position](#)” on page 17.
8. In the Solvents for LC Pump area, type the names of the solvents in the boxes for solvent bottles A and B.

Solvent bottle A typically contains LC/MS-grade water with 0.1% formic acid. Solvent bottle B typically contains LC/MS-grade acetonitrile with 0.1% formic acid.

9. If the OK button is unavailable, click **Test Connection** to download the new configuration settings to the data system computer.
10. Click **OK** to accept the settings and close the Thermo EASY-nLC Configuration dialog box.
11. Click **Done** to close the Thermo Foundation Instrument Configuration window.

You must close the Foundation application before opening the Xcalibur data system. The Xcalibur data system and the Thermo Foundation Instrument Configuration window cannot be open at the same time.

❖ **To change the loop volume, column setup, idle mixture, or idle flow rate**

1. From the instrument's touch screen, do the following:
  - a. Open the **Maintenance > Devices** page.
  - b. In the Devices list, select **EASY-nLC (HPLC)**, and then press the **Properties** tab.

The Properties view for the HPLC device on the Maintenance > Devices page opens (Figure 7).

**Figure 7.** EASY-nLC (HPLC) properties view (touch-screen software version 3.0 or later)

- c. Press the corresponding box to open the keypad and enter the appropriate numeric values for these parameters:
  - For Loop Volume, type the nominal volume of the sample loop installed on valve S (**5**, **20**, or **50** µL).
  - For Idle Flow Rate, type an appropriate value, in nL/min. When idle, the instrument pumps the specified solvent mixture at the specified flow rate.
  - For Idle Mixture %B, type a value from **0** to **100**.

## 2 Setting Up the Foundation Instrument Configuration

### Setting Up the EASY-nLC Configuration

- d. Select or clear these check boxes as appropriate: **One Column Setup** and **Load Speed Protection (LSP)**.

**Note** The LSP check box is available in the 3.0 or later version of the EASY-nLC touch-screen software.

- e. Click **Apply**.
2. In the EASY-nLC Configuration dialog box of the Foundation Instrument Configuration window, click **Test Connection**. Then verify the new settings.

### EASY-nLC Configuration Dialog Box Parameters

When you establish a connection between the EASY-nLC instrument and the data system computer, this dialog box displays the user-specified loop volume, idle flow rate, idle mixture, and column setup. You set these parameters from the EASY-nLC touch-screen software as described above in “To change the loop volume, column setup, idle mixture, or idle flow rate.”

Table 4 describes the parameters in the Thermo EASY-nLC Configuration dialog box.

**Table 4.** EASY-nLC Configuration dialog box parameters (Sheet 1 of 3)

Parameter	Description
<b>EASY-nLC Network Configuration</b>	
Use this area to specify the IP address for the EASY-nLC instrument.	
IP Address/Hostname	Specifies the IP address of the EASY-nLC instrument. This value must match the value stored by the instrument. <b>Tip</b> To check the IP address and host name stored by the instrument, press the company logo in the upper-left corner of the EASY-nLC touch screen.
User Name	Specifies the user name. <b>Note</b> The user name that you type in this box must match the user name for the person currently logged in to the instrument. The user name is listed in the lower-left corner of the EASY-nLC touch screen. For information about logging in to the EASY-nLC instrument, refer to the <i>EASY-nLC Series Getting Started Guide</i> .



**Table 4.** EASY-nLC Configuration dialog box parameters (Sheet 2 of 3)

Parameter	Description
Test Connection button	<p>Tests the Ethernet connection between the data system computer and the EASY-nLC instrument. If the connection is established, the status box to the right of the button turns green and contains this message: Connection OK.</p> <p>In addition, the LC device driver and instrument information appears in the box below the status bar.</p> <p><b>IMPORTANT</b> If the following message appears, exclude the Thermo EASY-nLC software from the McAfee virus scanner as described in <a href="#">“Excluding the EASY-nLC from the VirusScan Enterprise”</a> on page 5:</p> <p style="padding-left: 40px;">An established connection was aborted by the software in your host machine.</p> <p style="padding-left: 40px;">Contact your local IT administrator for assistance.</p>
<b>LC Device Driver and Instrument Information</b>	
LC Device Driver	Displays the LC device driver version.
LC Instrument	Displays the instrument type.
Driver Info	Displays the instrument’s touch-screen software version.
Serial Number	Displays the serial number that is listed on the instrument’s back panel.
Loop Volume	Displays the user-specified loop volume stored by the EASY-nLC instrument.
Idle Flow Rate	<p>Displays the user-specified idle flow rate that the instrument maintains at the end of an injection sequence.</p> <p>Entering a value of 0 deactivates this feature.</p> <p><b>Tip</b> Maintaining long-term emitter stability (with glass emitters) might require a constant solvent flow through the system.</p>
Idle Mixture	Displays the user-specified solvent composition (%B) that the instrument is to pump through the system at the end of an injection sequence.
Column Setup	Displays the user-specified selection. Displays One Column or Two Column.
IFC	Displays whether the intelligent flow control (IFC™) mode is enabled. The IFC mode is a user-selectable option in the touch-screen software version 2.8.
Max Pressure	Displays the maximum system pressure.
<b>Autosampler Configuration</b>	
Use this area to select the autosampler type, plate type, sample layout, and initial vial position.	
Autosampler	<p>Specifies the autosampler model:</p> <ul style="list-style-type: none"> <li>• ASC—Autosampler with refrigerated compartment and 6 extra vials</li> <li>• ASA—Autosampler with a cooled plate and without the 6 extra vials</li> </ul>

## 2 Setting Up the Foundation Instrument Configuration

Setting Up the EASY-nLC Configuration

**Table 4.** EASY-nLC Configuration dialog box parameters (Sheet 3 of 3)

Parameter	Description
Plate Installed	Specifies the plate type that you plan to use: 96 well plate, 384 well plate, or 6 × 8 vial plate.
Sample Layout	Displays the default sample layout (vial or microplate well positions) for an injection sequence.
Start Position	Displays the default initial sample position for an injection sequence.
Edit button	Opens the Sample Position Selector dialog box where you can select the sample layout and initial position for an Xcalibur sequence (see “ <a href="#">Setting Up the Sample Layout and Start Position</a> ” on page 17).
<b>Solvents for LC Pump</b>	
Use this area to describe the solvents that make up the gradient.	
A	Specifies the solvent in solvent bottle A.
B	Specifies the solvent in solvent bottle B.

## Setting Up the Sample Layout and Start Position

When you open the Sample Position Selector dialog box from the Thermo EASY-nLC Configuration dialog box, the Number of Samples to Select box and the Select button are unavailable.

When setting up the configuration options for the EASY-nLC instrument, use the Sample Position Selector dialog box to set up the sample layout and initial vial or microplate well position for an injection sequence.

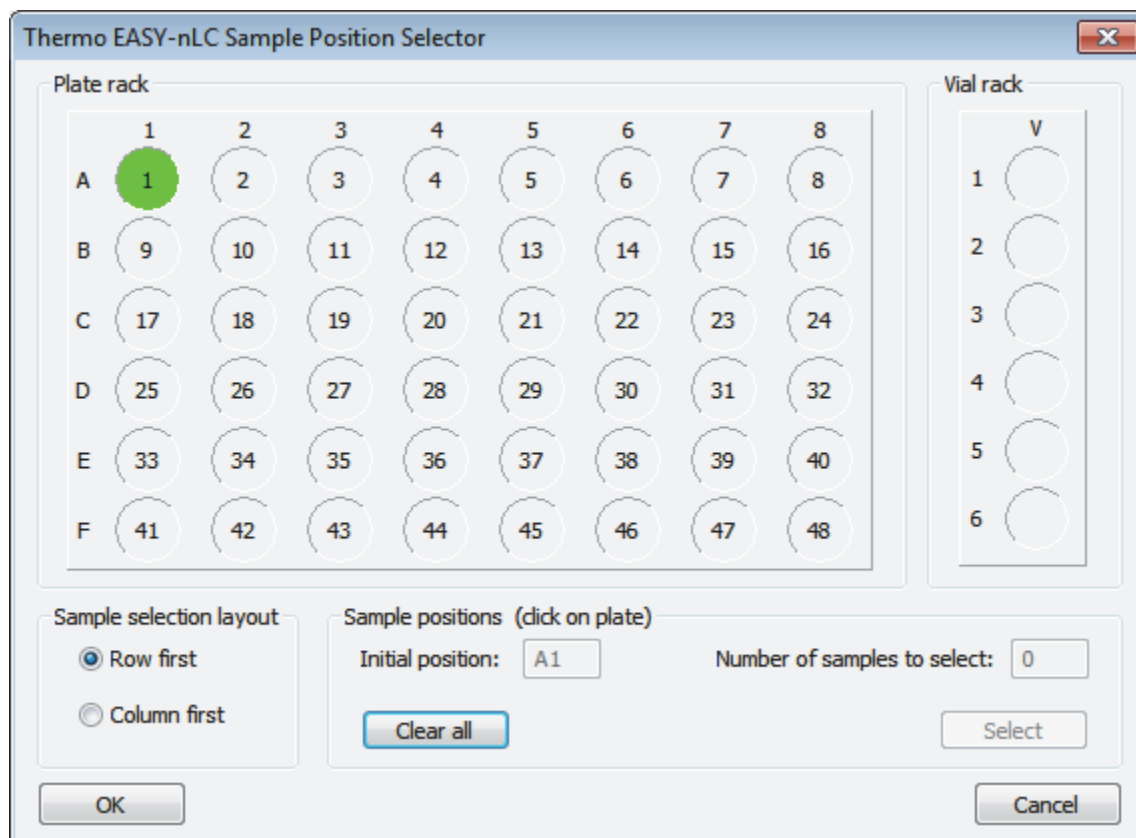
For information about using the Sample Position Selector dialog box to interactively select the vial or microplate well positions for an injection sequence, see [“Setting Up the Injection Positions with the Sample Position Selector”](#) on page 41.

### ❖ To set up the sample layout and start position for an Xcalibur injection sequence

1. In the Autosampler Configuration area, click **Edit**.

The Thermo EASY-nLC Sample Position Selector dialog box opens. The Number of Samples to Select box is unavailable (see [Figure 8](#)).

**Figure 8.** Sample Position Selector dialog box



## 2 Setting Up the Foundation Instrument Configuration

### Setting Up the Sample Layout and Start Position

2. In the Sample Positions area, click **Clear All**.
3. In the Plate Rack area, select the default initial sample position by clicking a position.
4. In the Sample Selection Layout area, select the **Row First** or **Column First** option.
5. Click **OK** to save your changes and exit the Thermo EASY-nLC Sample Position Selector dialog box.

**Note** You can also open the Thermo EASY-nLC Sample Position Selector dialog box by clicking Select Vials in the Samples area of the Xcalibur New Sequence Template dialog box (see [Figure 13](#) on [page 37](#)).

### Sample Position Selector Dialog Box Parameters

[Table 5](#) describes the parameters in the Sample Position Selector dialog box. When you access this dialog box from the EASY-nLC Configuration dialog box, only the Sample Position Layout and Initial Position parameters are available.

**Table 5.** Sample Position Selector dialog box parameters (Sheet 1 of 2)

Parameter	Description
<b>Plate Rack</b>	
Use this area to select or clear the sample positions.	
<b>Vial Rack</b>	
For an EASY-nLC instrument with an ASC model autosampler (standard in the EASY-nLC 1000 instrument), you can select or clear a sample position from the additional 6 vials.	
<b>Note</b> The additional vials are located on rack 1 and are called 1-V1 to 1-V6.	
<b>Sample Selection Layout</b>	
Row First	When you select the Row First option, the data system fills the sequence table with the sample positions row by row.
Column First	When you select the Column First option, the data system fills the sequence table with the sample positions column by column.
<b>Note</b> The sequence editor ignores the Sample Selection Layout option when you select the vial or well positions by clicking a set of samples in the Plate and Vial Rack areas.	

**Table 5.** Sample Position Selector dialog box parameters (Sheet 2 of 2)

<b>Parameter</b>	<b>Description</b>
<b>Sample Positions (click on plate)</b>	
Initial Position	Specifies the first vial or microplate well position in the sequence table.
Number of Samples to Select	Specifies the number of samples to add to the sequence table in addition to the initial vial position.
Clear All button	Clears all of the Plate and Vial Rack entries and the Initial Position entry.
Select button	Sequentially selects the number of vial or well positions specified in the Number of Samples to Select box. The selected vials or wells are highlighted in green in the Plate Rack and Vial Rack areas. The vials or wells are ordered sequentially according to the sample selection layout selection.
<b>Dialog box buttons</b>	
OK	Accepts the settings and closes the dialog box.
Cancel	Closes the dialog box without accepting the changes.



## Creating Xcalibur Instrument Methods

This chapter describes how to set up the instrument method parameters for the EASY-nLC instrument.

An Xcalibur instrument method (.meth) contains the chromatographic and data acquisition parameters for your LC/MS system. When creating new instrument methods, set up the parameters for all of the configured devices.

### Contents

- [Setting Up the EASY-nLC Instrument Method Parameters](#)
- [Saving Instrument Methods](#)

**Note** For information about setting up the instrument method parameters for the mass spectrometer, refer to the Help for the mass spectrometer.

## Setting Up the EASY-nLC Instrument Method Parameters

Use the Instrument Setup window of the Xcalibur data system to create instrument methods for your LC/MS system. To create an instrument method, enter the data acquisition settings for the mass spectrometer and the injection and chromatographic settings for the LC system.

### ❖ To open the Instrument Setup window

From the Xcalibur Home Page, do one of the following:

- Choose **GoTo > Instrument Setup**.

–or–

- Click the **Instrument Setup** icon in the Roadmap view of the Home Page.

The Instrument Setup window opens. The view bar contains icons for the configured devices.

### 3 Creating Xcalibur Instrument Methods

Setting Up the EASY-nLC Instrument Method Parameters

#### ❖ To set up the instrument method parameters for the EASY-nLC instrument

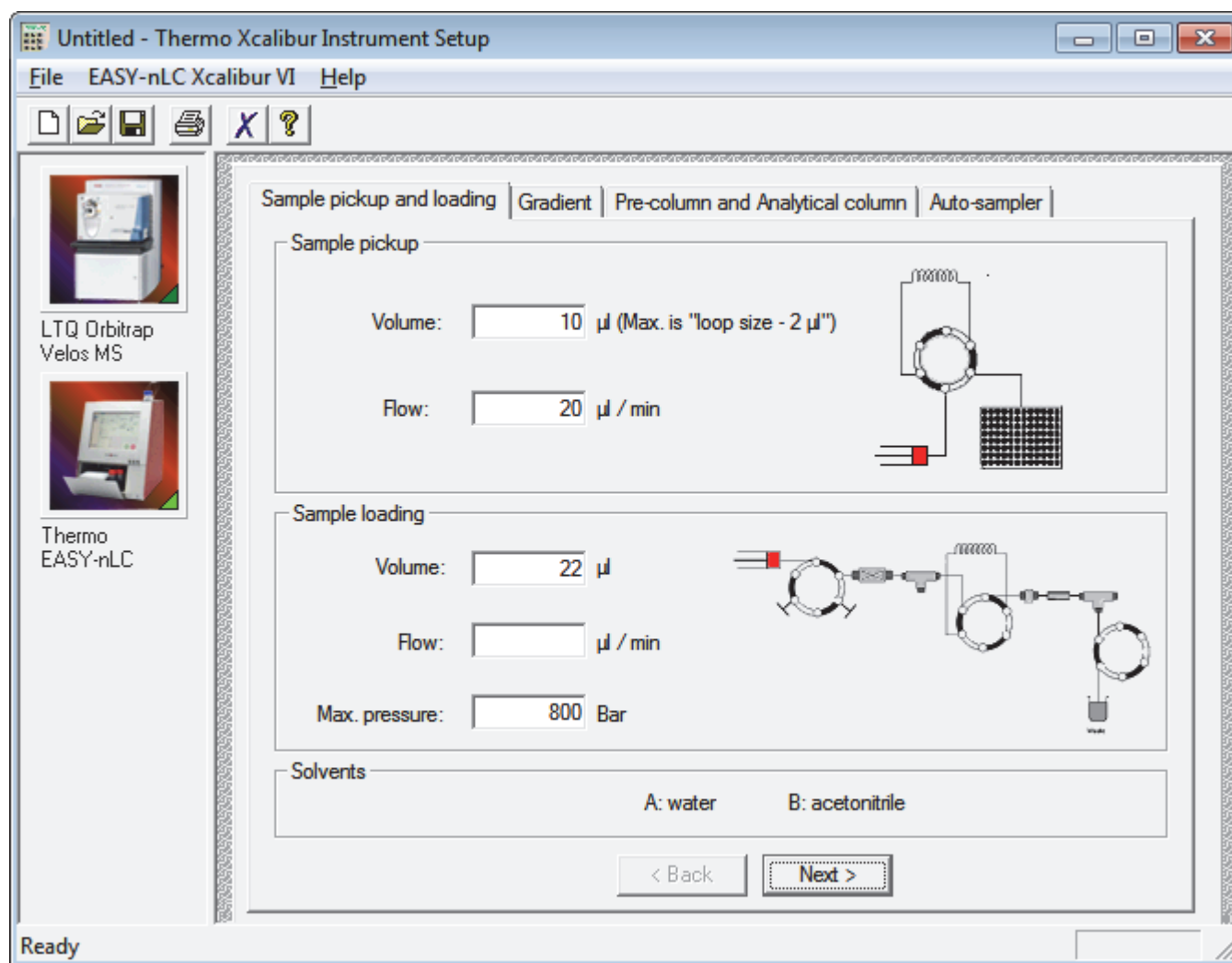
1. In the view bar of the Instrument Setup window, click the **Thermo EASY-nLC** icon.

The EASY-nLC view opens in the Instrument Setup window with the Sample Pickup and Loading page displayed (see Figure 9). At the bottom of the view, the Solvents area lists the solvents specified in the instrument configuration. To change the names of the mobile phase solvents, see “Setting Up the EASY-nLC Configuration” on page 10.

2. Enter the appropriate instrument method settings for the EASY-nLC instrument. Click **Next** to open the remaining three pages of the EASY-nLC view page by page.

Figure 9 shows the Sample Pickup and Loading page of the EASY-nLC view with typical sample pickup and loading settings for the EASY-nLC 1000 instrument and a sample in an aqueous matrix.

**Figure 9.** EASY-nLC view of the Instrument Setup window





For information about the pages of the EASY-nLC view, see these topics:

- “Sample Pickup and Loading Page” on page 23
- “Gradient Page” on page 25
- “Pre-Column and Analytical Column Page” on page 28
- “Auto-Sampler Page” on page 31

## Sample Pickup and Loading Page

Use the Sample Pickup and Loading page to specify the following:

- The sample volume that the autosampler withdraws (injection volume) from the vial or well position in the Xcalibur injection sequence and the flow rate at which the autosampler draws the sample into the sample loop.
- The solvent volume that pump A uses to push the sample out of the sample loop and onto the column assembly and the flow rate and maximum pressure for the sample loading step.

### ❖ To open the Sample Pickup and Loading page

1. Open the EASY-nLC view of the Instrument Setup window.
2. If the Sample Pickup and Loading page is not displayed, click the **Sample Pickup and Loading** tab.

## Sample Pickup and Loading Page Parameters

Table 6 describes the parameters on the Sample Pickup and Loading page of the EASY-nLC view of the Instrument Setup window.

**Table 6.** Sample Pickup and Loading page parameters

Parameter	Description
<b>Sample Pickup</b>	
Volume (µL)	<p>Specifies the sample volume that the autosampler withdraws from the vial or well position specified in the Xcalibur sequence.</p> <p>Maximum volume = <i>sample loop volume</i> – 2 µL</p> <p>The graphic on the Home &gt; Overview page (touch screen) displays the sample loop size.</p>
Flow (µL/min)	<p>Specifies the flow rate at which pump S draws the sample into the sample loop.</p> <p>Range: 0 to 40 µL/min. The flow must be greater than 0 when the sample pickup volume is greater than 0.</p> <p><b>Tip</b> For aqueous sample matrixes, set the flow rate to 20 µL/min.</p>
<b>Sample Loading</b>	
Volume (µL)	<p>Specifies the volume of solvent A that the instrument uses to push the sample out of the sample loop and onto the precolumn (two-column setup) or the analytical column (one-column setup).</p> <p>Typically, two times the sample pickup volume plus 2 µL is sufficient.</p> <p>Range: 0 to 137 µL</p> <p><b>IMPORTANT</b> The maximum allowable entry is 137 µL; however, for Xcalibur sequence runs, the <i>sample loading volume</i> plus the <i>precolumn equilibration volume</i> plus the <i>analytical column equilibration volume</i> must be less than 120 µL.</p> <p>See “Taking Corrective Action for Run-Time Errors” on page 45.</p>
Flow (µL/min)	<p>Specifies the flow rate at which pump A pumps solvent A through the instrument to push the sample out of the loop and onto the precolumn or analytical column.</p> <p>If the Flow box is empty, the instrument runs at the set pressure.</p> <p>Range: 0 to 100 µL/min</p>
Max Pressure (bar)	<p>Specifies the maximum pressure for the sample loading step.</p> <p>If the Max Pressure box is empty, the instrument runs at the set flow rate.</p> <p>EASY-nLC II range: 0 to 280 bar EASY-nLC 1000 range: 0 to 980 bar</p>
<p><b>Tip</b> If both the Max Pressure and Flow boxes are empty, the instrument runs at the maximum instrument pressure. Leave these boxes empty if you want the instrument to run at the highest possible flow rate that is allowed under the maximum instrument pressure limit.</p>	

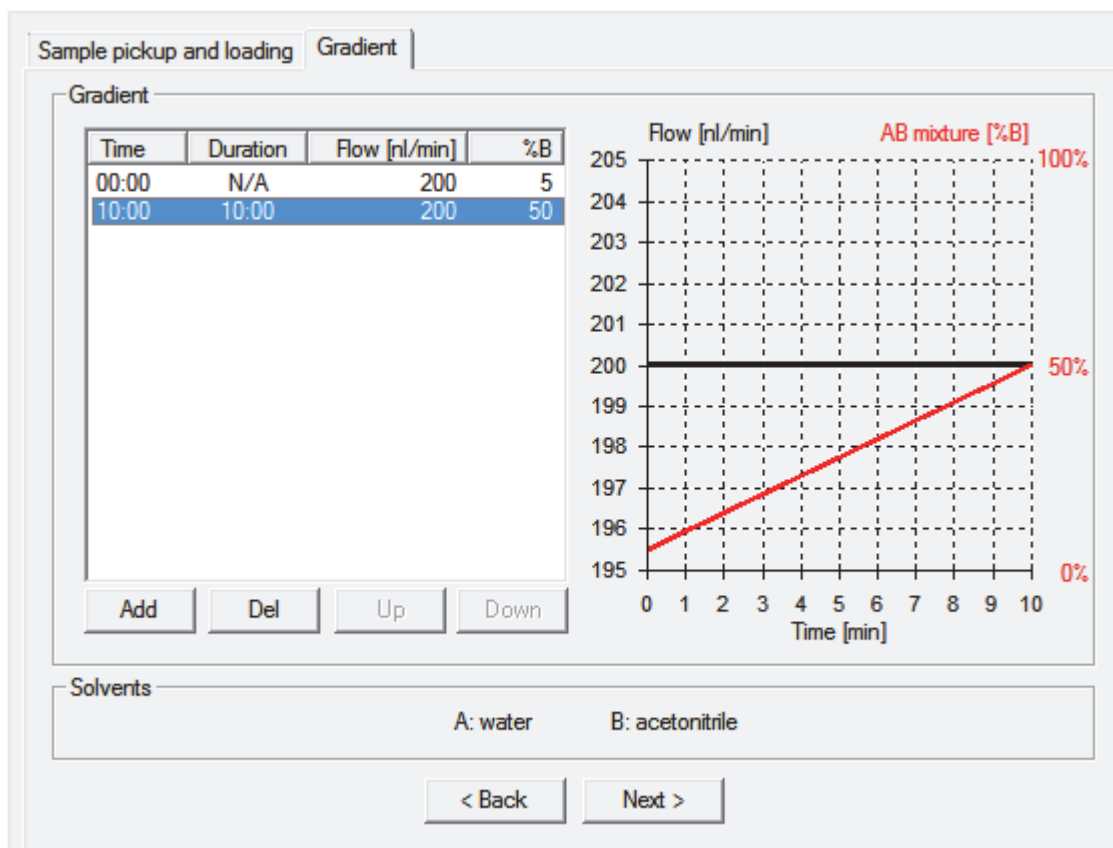
## Gradient Page

Use the Gradient page to set up the gradient program (see [Figure 10](#)).

### ❖ To open the Gradient page

1. Open the EASY-nLC view of the Instrument Setup window.
2. Click the **Gradient** tab. If you do not see the Gradient tab, click **Next**.

**Figure 10.** Gradient page



### ❖ To set up the gradient program

1. In the Time = 0.00 row, do the following:
  - In the Flow (nL/min) column, type a value from **1** to **2000**.
  - In the %B column, type a value from **0** to **100**.
2. To add an additional time point (table row), click **Add**.

### 3 Creating Xcalibur Instrument Methods

Setting Up the EASY-nLC Instrument Method Parameters

3. In rows 2 or higher of the gradient table, do the following:

a. In the Duration column, type a value from **0.01** to **9999.49**.

The value in the Duration column is the length of time that you want the instrument to take to reach the specified flow and %B conditions. When you type a value in the Duration column, the value in the Time column changes to the current Duration value plus the value in the previous row.

b. In the Flow (nL/min) column, type a value from **1** to **2000**.

c. In the %B column, type a value from **0** to **100**.

Both the flow rate and %B composition change linearly between time points.

4. Edit the table as follows:

- To delete a row, select the row and click **Del**.
- To reorder the rows as applicable, select the row and click **Up** or **Down**.

## Gradient Page Parameters

Table 7 describes the parameters on the Gradient page of the EASY-nLC view in the Instrument Setup window.

**Table 7.** Gradient page parameters

Parameter	Description
<b>Table</b>	
Time	Specifies the run time when the instrument reaches the conditions specified in the flow and %B columns.
Duration	Specifies the length of time between the previous and current time points. Range: 0.01 to 9999.59 minutes
Flow (nL/min)	Specifies the mobile phase flow rate for the current time point. Range: 1 to 2000 nL/min
%B	Specifies the percent composition of solvent B in the mobile phase for the current time point.
<b>Buttons</b>	
Add	Adds a row to the gradient program.
Del	Deletes the selected row from the gradient program.
Up	Moves the selected row up.
Down	Moves the selected row down.
<b>Graph</b>	
Flow (nL/min)	The <i>y</i> axis on the left side of the graph displays the flow scale. A black line represents the change in flow rate with time.
AB mixture (%B)	The <i>y</i> axis on the right side of the graph displays the percentage of solvent B. A red line represents change in %B with time.
Time (min)	The <i>x</i> axis displays the run time of the gradient program.

## Pre-Column and Analytical Column Page

Use the Pre-column and Analytical Column page to set up the column equilibration steps (see [Figure 11](#)).

#### ❖ To open the Pre-column and Analytical Column page

1. Open the EASY-nLC view of the Instrument Setup window.
2. Click the **Pre-column and Analytical Column** tab. If you do not see the Pre-column and Analytical Column tab, click **Next**.

**Figure 11.** Pre-column and Analytical Column page

Sample pickup and loading | Gradient | Pre-column and Analytical column

Pre-column equilibration

Volume:  µl

Flow:  µl / min

Max. pressure:  Bar

Analytical column equilibration

Volume:  µl

Flow:  µl / min

Max. pressure:  Bar

Solvents

A: water      B: acetonitrile

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## Pre-Column and Analytical Column Page Parameters

Table 8 describes the parameters on the Pre-column and Analytical Column page of the EASY-nLC view of the Instrument Setup window.

**Note** For information about determining the appropriate flow rate for the precolumn and analytical column equilibration steps, refer to the *EASY-nLC Series Getting Started Guide*.

**Table 8.** Pre-column and Analytical Column page parameters (Sheet 1 of 2)

Parameter	Description
<b>Pre-column Equilibration</b>	
Volume (µL)	Specifies the volume of solvent A that pump A pushes through the precolumn to waste during the precolumn equilibration step.  Range: 0 to 137 µL  <b>IMPORTANT</b> The maximum allowable entry is 137 µL; however, for Xcalibur sequence runs, the <i>sample loading volume</i> plus the <i>precolumn equilibration volume</i> plus the <i>analytical column equilibration volume</i> must be less than 120 µL.
Flow (µL/min)	Specifies the flow rate for the precolumn equilibration step. If the Flow box is empty, the instrument runs at the set pressure.  Range: 0 to 100 µL/min
Max Pressure (bar)	Specifies the maximum pressure for the precolumn equilibration step. If the Max Pressure box is empty, the instrument runs at the set flow rate.  EASY-nLC II range: 0 to 280 bar EASY-nLC 1000 range: 0 to 980 bar
<b>Tip</b> If both the Max Pressure and Flow boxes are empty, the instrument runs at the maximum instrument pressure. Leave these boxes empty if you want the instrument to run at the highest possible flow rate that is allowed under the maximum instrument pressure limit.	

### 3 Creating Xcalibur Instrument Methods

Setting Up the EASY-nLC Instrument Method Parameters

**Table 8.** Pre-column and Analytical Column page parameters (Sheet 2 of 2)

Parameter	Description
<b>Analytical Column Equilibration</b>	
Volume (μL)	<p>Specifies the volume of solvent A that pump A pushes through the analytical column during the analytical column equilibration step.</p> <p>Range: 0 to 137 μL</p> <p><b>IMPORTANT</b> The maximum allowable entry is 137 μL; however, for Xcalibur sequence runs, the <i>sample loading volume</i> plus the <i>precolumn equilibration volume</i> plus the <i>analytical column equilibration volume</i> must be less than 120 μL.</p> <p>See <a href="#">“Taking Corrective Action for Run-Time Errors”</a> on page 45.</p>
Flow (μL/min)	<p>Specifies the flow rate for the analytical column equilibration step. If the Flow box is empty, the instrument runs at the set pressure.</p> <p>Range: 0 to 3 μL/min</p>
Max Pressure (bar)	<p>Specifies the maximum pressure for the analytical column equilibration step.</p> <p>EASY-nLC II range: 0 to 280 bar EASY-nLC 1000 range: 0 to 980 bar</p>
<p><b>Note</b> If both the Max Pressure and Flow boxes are empty, the instrument runs at the maximum instrument pressure. Leave these boxes empty if you want the instrument to run at the highest possible flow rate that is allowed under the maximum instrument pressure limit.</p>	



## Auto-Sampler Page

Use the Auto-sampler page to set up the wash step for the needle and sample loop (see [Figure 12](#)).

### ❖ To open the Auto-sampler page

1. Open the EASY-nLC view of the Instrument Setup window.
2. Click the **Auto-sampler** tab. If you do not see the Auto-sampler tab, click **Next**.

**Figure 12.** Auto-sampler page

Sample pickup and loading | Gradient | Pre-column and Analytical column | **Auto-sampler**

Auto-sampler wash

Standard

Flush volume:  µl

Custom

Order	Source	Volume [µl]	Cycles
-------	--------	-------------	--------

Add Del Up Down

Note: Max. vol. is "loop size + 8 µl". Wash bottle is no. 4.

Solvents

A: water B: acetonitrile

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### ❖ To set up the standard autosampler wash step

1. Select the **Standard** option.
2. In the Flush Volume (µL) box, type the volume of solvent A (from wash bottle 3) that the autosampler uses to flush the needle and the sample loop.

The range depends on the sample loop size (see [Table 9](#) on [page 33](#)).

#### ❖ To set up a custom wash program

1. Select the **Custom** option.

The custom wash table becomes available.

2. For each row that you want to add to the program, click **Add**.

You can select a different wash solvent for each row.

3. Edit each row as follows:

- In the Source column, select the wash solvent source: **Bottle 1**, **Bottle 2**, or **Bottle 3**.

**IMPORTANT** The EASY-nLC tray compartment holds up to four wash bottles:

- Bottles 1 and 2 in positions W1 and W2 can contain custom wash solvents.
- Bottle 3 in position W3 must contain solvent A.
- Bottle 4 in position W4 must contain the needle wash insert.

During a custom wash program, the autosampler does the following:

1. Empties the contents of pump S into the needle wash bottle (W4).
2. Performs the steps in the custom wash program. During each cycle, the autosampler draws solvent from the specified bottle, and then ejects the solvent into the needle wash bottle (W4).
3. Performs a final wash cycle with a preset volume of solvent A from bottle 3 (W3).
4. Refills pump S with solvent A from bottle 3 (W3) to be ready for the next injection.

- In the Volume ( $\mu\text{L}$ ) column, type a volume from **0** to the *sample loop size* plus 8  $\mu\text{L}$ .

If you enter a volume that is higher than 58  $\mu\text{L}$ , an Invalid Method Parameter message box appears and the program resets the volume to the maximum allowed.

- In the Cycles column, type a value from **0** to **10**.

## Auto-sampler Page Parameters

Table 9 describes the parameters on the Auto-sampler page of the EASY-nLC view of the Instrument Setup window.

**Table 9.** Auto-sampler page parameters (Sheet 1 of 2)

Parameter	Description
<b>Standard</b>	
Standard option	Selecting this option enables the standard wash option.
Flush Volume (µL)	Specifies the flush volume that pump S is to push through the sample loop.  Range for the 20 µL sample loop: 30.25 to 100.75 µL Range for the 50 µL sample loop: 60.26 to 70.75 µL
<b>Custom</b>	
Custom option	Selecting this option enables the custom wash program.  For information about the steps that the autosampler performs during a custom wash program, see <a href="#">“To set up a custom wash program”</a> on page 32.
<b>Custom wash table columns</b>	
Order	Defines the order of the custom wash steps.
Source	Specifies the wash solvent source for the current wash step.  Selections: Bottle 1, Bottle 2, or Bottle 3  <b>Note</b> Bottles 1 and 2 contain custom wash solvents. Bottle 3 contains solvent A.
Volume (µL)	Specifies the wash solvent volume for each wash cycle.  Maximum allowed volume at run time: <i>Sample loop size</i> plus 8 µL.  <b>Note</b> The Xcalibur method editor for the EASY-nLC instrument does not know the configured sample loop size. When you submit a sequence with the instrument method, Xcalibur acquisition service validates the custom wash volumes against the configured sample loop size. To prevent solvent from being drawn into pump S, the program limits the volume to the <i>sample loop size</i> plus 8 µL.  For information about changing the sample loop size on the Maintenance > Devices page of the touch-screen software, see <a href="#">“To change the loop volume, column setup, idle mixture, or idle flow rate”</a> on page 13.
Cycles	Specifies the number of wash cycles for the wash step.  Range: 1 to 10  <b>Note</b> For each cycle, the autosampler draws the specified volume of wash solvent from the specified source into the needle, and then ejects the wash solvent into wash bottle 4 (W4).

**Table 9.** Auto-sampler page parameters (Sheet 2 of 2)

Parameter	Description
<b>Buttons</b>	
Add	Adds a row to the wash program.
Del	Deletes the selected row from the wash program.
Up	Moves the selected row up.
Down	Moves the selected row down.

## Saving Instrument Methods

After you set up the instrument method parameters for the LC/MS system, save the instrument method to an appropriate directory.

### ❖ To save your current instrument method

1. Choose **File > Save As**.

The Save As dialog box opens.

2. In the File Name box, type a name for the new instrument method.

3. Click **Save**.

The File Summary Information dialog box opens.

4. Click **OK**.

# Creating and Running Sequences

This chapter describes how to create and run sequences in the Xcalibur Sequence Setup view.

## Contents

- [Creating Sequences](#)
- [Setting Up the Injection Positions with the Sample Position Selector](#)
- [Running Sequences](#)
- [Taking Corrective Action for Run-Time Errors](#)

## Creating Sequences

In the Xcalibur data system, the table that contains the sample injection information is called a sequence. In addition to the data file name for each injection, the sequence must contain the vial or microplate well positions of the samples (unknowns, calibration standards, quality control check standards, and so on) in the sample set, the instrument method to be used, and the injection volume. You can specify a different instrument method for each injection.

**Tip** You cannot change the tray type for the EASY-nLC instrument in the Sequence Setup view of the Xcalibur Home Page. If you choose **Change > Tray Name** from the menu bar, the following Home Page message appears:

The configured autosampler does not offer any selection of trays.



To change the tray type, close the Xcalibur data system. Then, open the EASY-nLC Configuration dialog box as described in “[Setting Up the EASY-nLC Configuration](#)” on [page 10](#) and select the appropriate tray type.

## 4 Creating and Running Sequences

### Creating Sequences

#### ❖ To create sequences in the Xcalibur data system


1. Do one of the following to open the Home Page – Sequence Setup view:

- From the Home Page – Roadmap view, click the **Sequence Setup** icon, .
- From any Home Page view, choose **View > Sequence Setup View**.
- From the Instrument Setup window, click the **Home Page** icon, , and then choose **View > Sequence Setup View**.

2. To open the New Sequence Template dialog box, do one of the following:

- Choose **File > New**.

–or–

- Click the **New Sequence** icon, , on the toolbar.

The New Sequence Template dialog box opens (see [Figure 13](#)).

Figure 13. New Sequence Template dialog box

**General**

Base File Name: test Starting Number: 1

Path: C:\Xcalibur\Data\ Browse...

Instrument Method: C:\Xcalibur\methods\easy Browse...

Processing Method: Browse...

Calibration File: Browse...

**Samples**

Number of Samples: 5 Tray Type: 6x8 vials

Injections per Sample: 1 Initial Vial Position: A1  Re-Use Vial Positions

Base Sample ID: Select Vials... Cancel Selection

**Bracket Type**

None  Open  Non-Overlapped  Overlapped

**Calibration**

Add Standards

Number of brackets: 1

Injections per Level: 1

Add Blanks

Fill in Sample ID for Standards

**QC**

Add QCs

After First Calibration Only

After Every Calibration

Add Blanks

Fill in Sample ID for QCs

OK Cancel Save As Default Help

For the EASY-nLC instrument, you cannot change the tray type in the Sequence Setup view. Tray Type is a read-only parameter.

## 4 Creating and Running Sequences

### Creating Sequences

3. In the General area, do the following:
    - Type the base file name for the data files.
    - Type a starting number for the value that the data system appends to the base file name.
    - Browse to select the directory location for the data files in the Path box.
    - Browse to select the instrument method in the Instrument Method box.
  4. Set up the vial or well positions by doing one of the following:
    - To use the text boxes in the Samples area of the New Sequence Template dialog box, go to [step 5](#).
- or–
- To use the Sample Position Selector dialog box, go to [“Setting Up the Injection Positions with the Sample Position Selector”](#) on [page 41](#).

**Tip** You can set up the sample positions for a sequence in three ways:

- In the Samples area of the New Template Sequence dialog box, type the number of samples in the Number of Samples box and the starting vial position in the Initial Vial Position box.
- In the Sample Position Selector dialog box, do one of the following:
  - Type the number of samples in the Number of Samples to Select box, and then click **Select** (see [Figure 15](#) on [page 41](#)).
  - Click the positions in the Plate Rack and Vial Rack areas. Use this method to set up noncontiguous, nonsequential, or noncontiguous and nonsequential positions.



5. Set up the vial or well positions by using the Samples area of the New Sequence Template as follows:

- In the Number of Samples box, type the number of samples for the injection sequence.
- In the Injections per Sample box, type the number of injections that you want the autosampler to make for each sample.
- For replicate sample injections, do the following:
  - Select the **Re-Use Vial Positions** check box if you want the autosampler to make replicate injections from the same sample vial or well. Make sure that the vial or well contains a sufficient volume.
  - Clear the **Re-Use Vial Positions** check box if you want the autosampler to make replicate injections from different sample vials or wells. Load the autosampler with a sufficient number of replicate samples.
- In the Initial Vial Position box, type the vial position for the first sample injection. For alphanumeric entries, use uppercase letters (see [“Taking Corrective Action for Run-Time Errors”](#) on page 45).

**Note** The sequence editor fills in the Positions column of the sequence table with contiguous vial or well locations based on the initial position, the number of samples, the number of replicate injections, and the sample selection layout option selected in the Foundation Instrument Configuration window.

- Selecting the Row First option in the instrument configuration application prompts the sequence editor to fill in the positions row by row (for example, A1–A8, B1–B8, and so on).
- Selecting the Column First option in the instrument configuration application prompts the sequence editor to fill in the positions column by column (for example, A1–F1, A2–B2, and so on).
- Clearing the Re-Use Vials check box prompts the sequence editor to enter an additional vial or well position for each replicate.

6. To set up the remaining parameters in the New Sequence Template dialog box, refer to the Sequence Setup view Help.

## 4 Creating and Running Sequences

### Creating Sequences

7. Click **OK** to create the sample sequence.

The sequence information appears in the Sequence Setup view (see [Figure 14](#)).

**Figure 14.** Sequence Setup view on the Xcalibur Home Page

	File Name	Path	Inst Meth	Position	Inj Vol
1	AssayC1	C:\Xcalibur\Data\	C:\Xcalibur\methods\Protein Assay	A1	-1.0
2	AssayC2	C:\Xcalibur\Data\	C:\Xcalibur\methods\Protein Assay	A2	-1.0
3	AssayC3	C:\Xcalibur\Data\	C:\Xcalibur\methods\Protein Assay	A3	-1.0
4	AssayC4	C:\Xcalibur\Data\	C:\Xcalibur\methods\Protein Assay	A4	-1.0
*					0.0

When the Inj Vol column lists a value of -1, the instrument uses the Sample Pickup volume that is specified in the instrument method.

8. Check the following:

- The vial or well values in the Position column. Make sure that the entries are valid and use uppercase letters.
- The numeric value in the Inj Vol column. When the value is -1, the data system uses the sample pickup volume specified in the instrument method.

**IMPORTANT** The sequence table contains a column called Inj Vol (injection volume). If the column does not appear, choose **Change > Column Arrangement** and add the Inj Vol column to the displayed columns.

To ensure that the injection volume specified in the instrument method file is used as intended, the default value in the Inj Vol column is set to -1, which is an invalid injection volume. When the Xcalibur data system detects the invalid value during a run, it automatically takes the actual injection volume from the corresponding instrument method file, overriding the -1 with the correct value.

During a run, the autosampler draws the specified injection volume (sample pickup volume) into the sample loop. The system then flushes the sample out of the sample loop and onto the LC column with the specified sample loading volume. Specifying an insufficient loading volume can result in blank injections or poor injection reproducibility because the system is either not loading or only partially loading the sample onto the LC column.

Changing the sample pickup volume by specifying a different value in the Inj Vol column of the sequence does not change the sample loading volume specified in the instrument method. To prevent incompatible settings, avoid using the Inj Vol column to vary the injection volume. Instead, create different instrument methods, where each instrument method contains compatible settings for the sample loading and sample pickup volumes (see [“Sample Pickup and Loading Page Parameters”](#) on page 24). You can use a different instrument method for each sequence row.

## Setting Up the Injection Positions with the Sample Position Selector

For information about the parameters in the Sample Position Selector dialog box, see [Table 5](#) on [page 18](#). The functionality of the Sample Position Selector dialog box depends on where you access the dialog box:

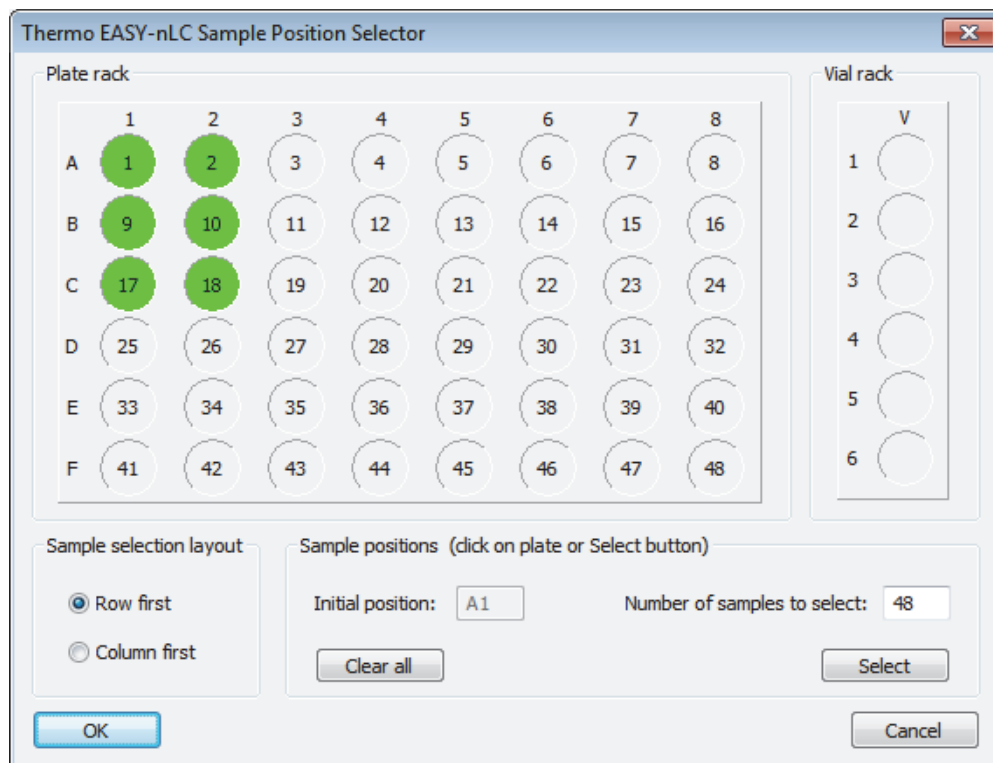
- When you open the Sample Position Selector dialog box from the EASY-nLC Configuration dialog box of the Thermo Foundation > Instrument Configuration window, use the dialog box to set up the sample layout and initial vial or microplate well position for an injection sequence (see “[Setting Up the Sample Layout and Start Position](#)” on [page 17](#)).
- When you open the Sample Position Selector dialog box from the New Sequence Template dialog box in the Xcalibur Sequence Setup view, use the dialog box to interactively set up the vial or microplate well positions for the injection sequence as described in the following procedure.

### ❖ To open the Sample Position Selector dialog box from the New Sequence Template dialog box and set up the sample positions

1. Click **Select Vials** in the Samples Area (see [Figure 13](#) on [page 37](#)).

The Thermo EASY-nLC Sample Position Selector dialog box opens (see [Figure 15](#)). The rows for the 6 × 8 vial tray are labeled A–F, and the columns are labeled 1–8. The six additional vials in the Vial Rack area are numbered 1–V1 to 1–V6.

**Figure 15.** Thermo EASY-nLC Sample Position Selector dialog box



## 4 Creating and Running Sequences

Setting Up the Injection Positions with the Sample Position Selector

2. Follow one of these procedures:
  - To select vials or wells by using the Plate Rack and Vial Rack areas
  - To select contiguous vials or wells in sequential order by using the Select button
3. To set up the remaining parameters in the New Sequence Template dialog box, refer to the Sequence Setup view Help.
4. Click **OK** to create the sample sequence.

The sequence information appears in the Sequence Setup view on the Xcalibur Home Page (see [Figure 14](#) on [page 40](#)).

### ❖ To select vials or wells by using the Plate Rack and Vial Rack areas

1. In the Sample Positions (Click on Plate or Select Button) area, click **Clear All**.

This clears the Initial Position box and any selected positions on the Plate Rack and the Vial Rack.

2. Select contiguous or noncontiguous vials or wells in a sequential or nonsequential order by clicking the positions on the Plate Rack and Vial Rack areas in the order that you want the samples to be injected.

Clicking a position turns it green. The first position that you click becomes the initial position in the sequence table. The sequence editor ignores the option selected in the Sample Selection Layout area and fills in the remaining rows in the order of your selections.

3. Click **OK** to return to the New Sequence Template dialog box.

### ❖ To select contiguous vials or wells in sequential order by using the Select button

1. In the Sample Positions (Click on Plate or Select Button) area, click **Clear All**.

This clears the Initial Position box and any selected positions on the Plate Rack and the Vial Rack.

2. In the Sample Selection Layout area, select either the **Row First** or the **Column First** option.
3. In the Number of Samples to Select box, type the number of sample vials or wells.
4. Click **Select**.

The Plate Rack area displays the selected positions in green.



5. Click **OK** to return to the New Sequence Template dialog box.

## Running Sequences


After you create a sequence with the appropriate data file names, instrument methods, and injection positions, you can run one or more rows of the sequence from the Sequence Setup view.

### ❖ To run a single sample, a range of samples, or the entire sequence set

1. In the Sequence Setup view, do one of the following:

- To inject a single sample, select the specific row in the sequence table. Then choose **Actions > Run This Sample**, or click the **Run Sample** icon, , in the Sequence Editor toolbar.
- To inject a range of samples, select the samples that you want to inject in the sequence table. Then choose **Actions > Run Sequence**, or click the **Run Sequence** icon, , in the Sequence Editor toolbar.

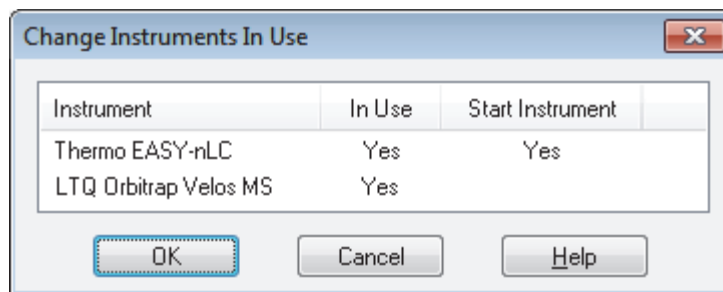
–or–

- To inject the entire sequence, choose **Actions > Run Sequence**, or click the **Run Sequence** icon, , in the Sequence Editor toolbar.

One of the following actions occurs:

- If the data system detects a new instrument configuration, the Change Instruments In Use dialog box opens (see [Figure 16](#)). Go to [step 2](#).
- If the instrument configuration has not changed since the last sequence run, the Run Sequence dialog box opens (see [Figure 17](#)). Go to [step 3](#).

**Figure 16.** Change Instruments In Use dialog box

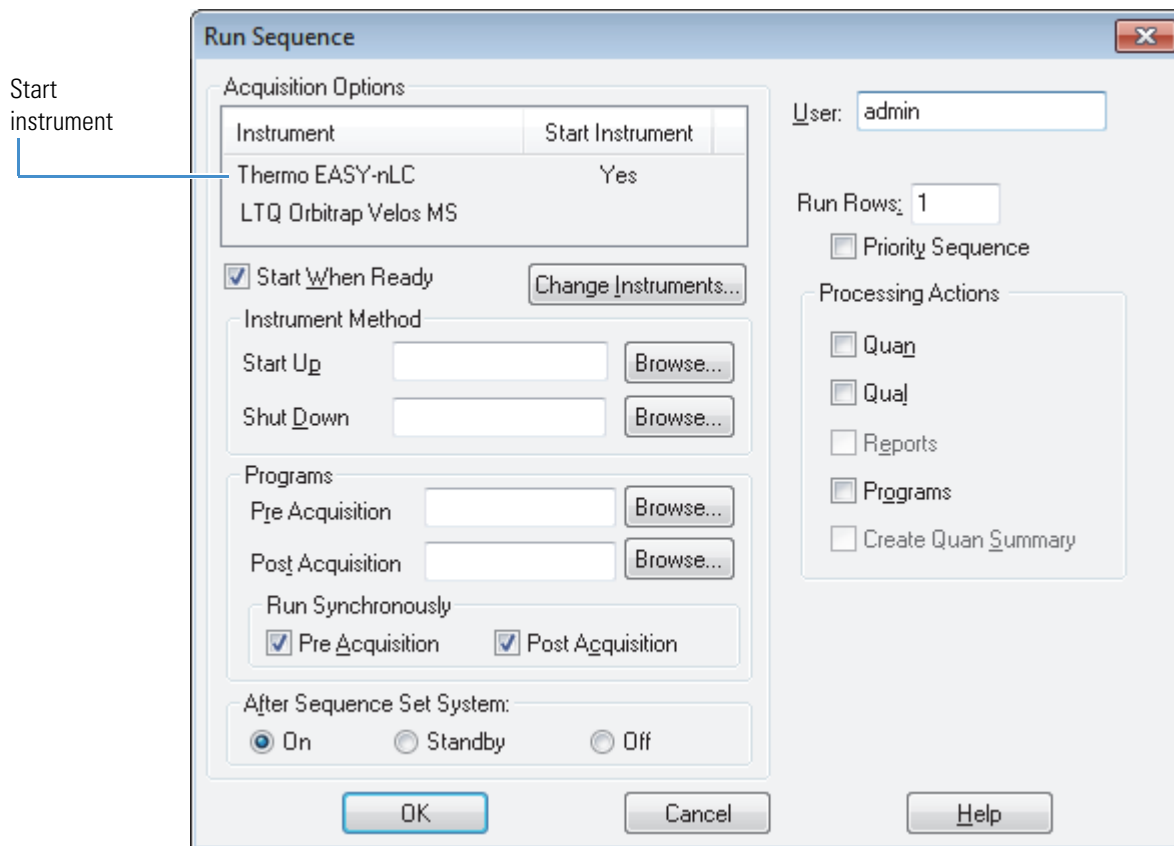


2. If the Change Instruments In Use dialog box opens, do one of the following:

- If the EASY-nLC instrument is not listed as the Start Instrument, click the EASY-nLC row in the Start Instrument column and select **Yes**. Then click **OK** to accept the new setting and close the dialog box.
- If the EASY-nLC instrument is listed as the Start Instrument, click **OK** to close the dialog box.

The Run Sequence dialog box opens (see [Figure 17](#)).

Figure 17. Run Sequence dialog box



3. Check whether the EASY-nLC instrument is listed as the start instrument in the Acquisition Options area.

The Start Instrument column displays Yes for the instrument that triggers data acquisition. When the EASY-nLC instrument begins its gradient program, it signals the MS detector to begin data acquisition. This signal is transmitted through the contact closure cable.

4. Depending on whether the EASY-nLC instrument is listed as the start instrument, do one of the following:
  - If the EASY-nLC instrument is listed as the start instrument, go to [step 5](#).
  - If the EASY-nLC instrument is not listed as the start instrument, make it the start instrument as follows:
    - a. Click **Change Instruments**.  
The Change Instruments In Use dialog box opens.
    - b. Click the Thermo EASY-nLC row in the Start Instrument column.  
The blank cell now displays Yes.
    - c. Click **OK** to accept the new setting and close the dialog box.

5. In the Run Sequence dialog box, do the following:
  - a. Make sure that the **Start When Ready** check box is selected.
  - b. Check the range in the Run Rows box.
  - c. Click **OK** to submit the sample run to the acquisition queue.

## Taking Corrective Action for Run-Time Errors

The method editor in the EASY-nLC view of the Instrument Setup window checks each parameter entry; however, it does not check for volume conflicts among the entries.

If you have specified an invalid volume for sample loading, column equilibration, or the gradient program, an error message appears when you attempt to run samples with the instrument method from the Sequence Setup view.

Table 10 describes the corrective action for common run-time errors that are caused by incompatible settings in the Xcalibur instrument method.

**Table 10.** Run-time errors caused by incompatible settings in the instrument method

Message	Corrective action
Sample pickup flow must be greater than 0 when pickup volume is not 0.	Enter a nonzero flow rate in the Flow box in the Sample Pickup area on the Sample Pickup and Loading Page.
Solvent A consumption in the method is greater than 120.0 during precolumn, analytical column, and sample loading.	Modify the method so that the total volume for the sample loading, the precolumn equilibration, and the analytical column equilibration steps is less than 120 µL.
Gradient: Solvent A consumption is greater than 132 µL.	Check the gradient table. Reduce the volume of solvent A that is used to produce the gradient.
Autosampler wash flush volume out of range:137 (30.25<—>100.75)	For a 20 µL sample loop, enter a volume from 30.25 to 100.75 µL in the Flush Volume box in the Standard Wash area of the Auto-sampler page.

Table 11 lists common run-time errors caused by incorrect entries in the Xcalibur sequence.

**Table 11.** Run-time errors caused by incorrect entries in the sequence table

Message	Remedy
EASY-nLC devices reported an error during the Prepare for Run command.	Reduce the injection volume (sample pickup volume).  The sample pickup volume cannot be greater than the sample loop size minus 2 µL, or 18 µL for a 20 µL sample loop.







## Viewing the Run Status in the Information View

Use the Status page of the Xcalibur Information view to view the run status of the EASY-nLC instrument.

### ❖ To monitor the run status for the EASY-nLC instrument from the Information view

1. Open the Xcalibur Home Page window as follows:
  - From the Processing Setup window, choose **GoTo > Xcalibur Home Page**.
  - From the Instrument Setup window, click the **Return to Home Page** icon, , on the toolbar.

**Note** The Qual and Quan Browser windows open in front of the Home Page window.

2. If the Information view is hidden, click the **Info View** icon, , on the View toolbar (see [Figure 18](#) on [page 48](#)).
3. During a run, click the Thermo EASY-nLC expand/collapse icon to display the EASY-nLC run progress and the instrument component readbacks on the bottom portion of the Status page.
4. If the MS detector remains in the Waiting for Contact Closure state during a run, do the following:
  - Check the contact closure connection between the contact closure pins on the back panel of the EASY-nLC instrument and the MS detector's Start In pins.
  - Check the MS detector selection (with the part number for the appropriate contact closure cable) on the Configuration > Connections page of the EASY-nLC touch-screen application.

[Figure 18](#) shows the Status page for an LC/MS system with an EASY-nLC instrument and an LTQ XL™ MS detector. The EASY-nLC instrument is loading the sample onto the column assembly and the MS detector is waiting for the contact closure signal from the EASY-nLC instrument.

Figure 18. Status page of the Information view

Info view icon

Expand/collapse icon

Run-time steps for the EASY-nLC instrument

During a run, the MS detector remains in the Waiting for Contact Closure state until the LC instrument sends the start data acquisition signal.

Status of the instrument components

Thermo Xcalibur Roadmap

File Actions View Tools GoTo Help

Status Acquisition Queue

Run Manager

- Check Devices
- Sequence:
- Sample Name:
- Working On:
- Position:
- Raw File:
- Inst. Method:
- Thermo EASY-nLC
  - Running
- LTQ XL MS
  - Waiting for Contact Closure

admin@172.16.0.103 (LC-000409)

Initialize system  
 Prealign A+B  
 Refill A  
 Refill B  
 Equilibrate precolumn  
 Equilibrate analytical column  
 Pickup sample  
**Load sample**  
 Prepare gradient  
 Run gradient  
 Autosampler wash + refill S

**Pump A:**  
 Desired flow [nl/min] : 40000.00  
 Position [µl] : 132.69  
 Pressure [bar] : 56.70  
 Real flow [nl/min] : 15000.00

**Pump B:**  
 Desired flow [nl/min] : 0.00  
 Position [µl] : 137.00  
 Pressure [bar] : -0.10  
 Real flow [nl/min] : -181.17

**Pump S:**  
 Desired flow [nl/min] : 0.00  
 Position [µl] : 112.27  
 Pressure [bar] : 0.30

**Valve A:**  
 Position : 1-6

**Valve B:**  
 Position : CENTER

**Valve S:**  
 Position : 1-6

**Valve W:**  
 Position : 1-2

**Autosampler:**  
 Temp [°C]: : 28.44



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