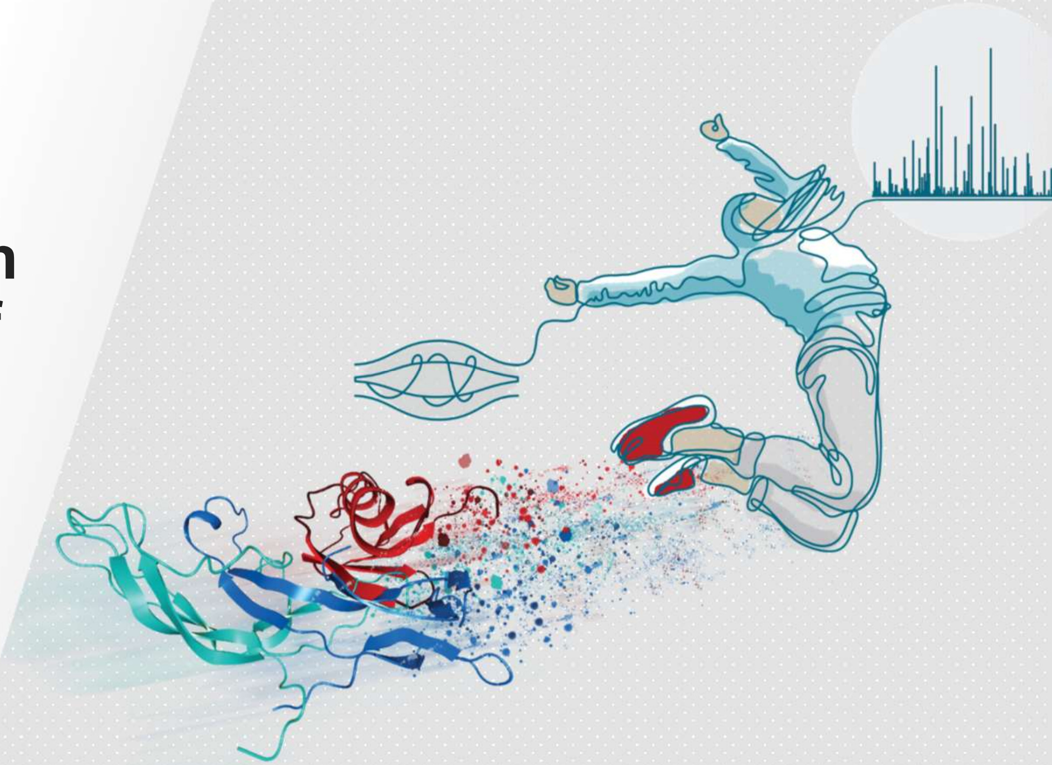
Democratizing proteomics through standardization and automation of sample preparation

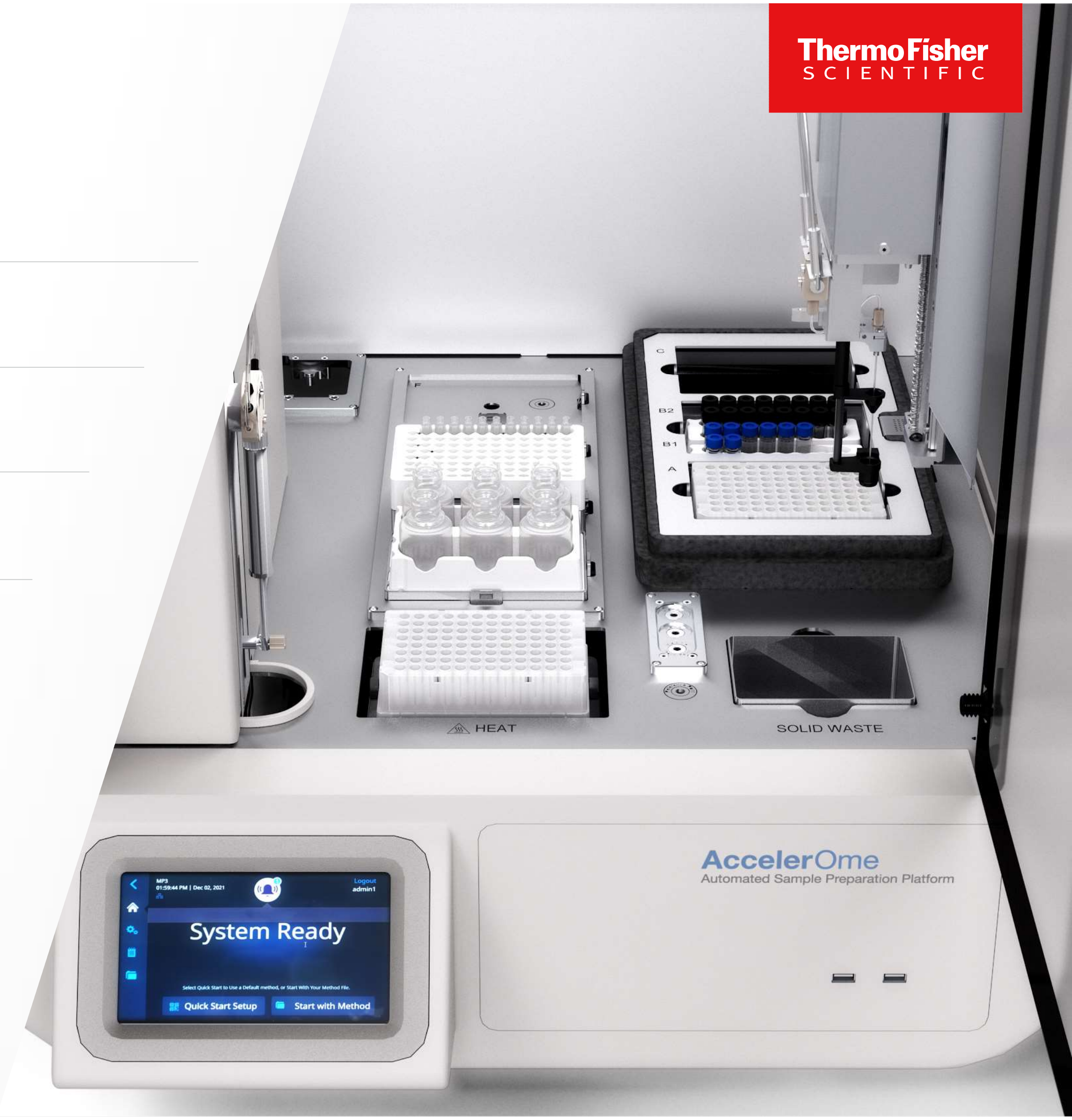


The world leader in serving science

Kristan.Bahten@thermofisher.com | 5 June 2022

Agenda

1. Challenges of proteomics sample preparation
2. Concept and design
3. Thermo Scientific AccelerOme automated sample preparation platform
4. Increasing productivity
5. Flexible workflows
6. Integration with Orbitrap LC-MS workflow
7. Summary



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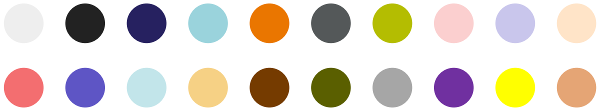
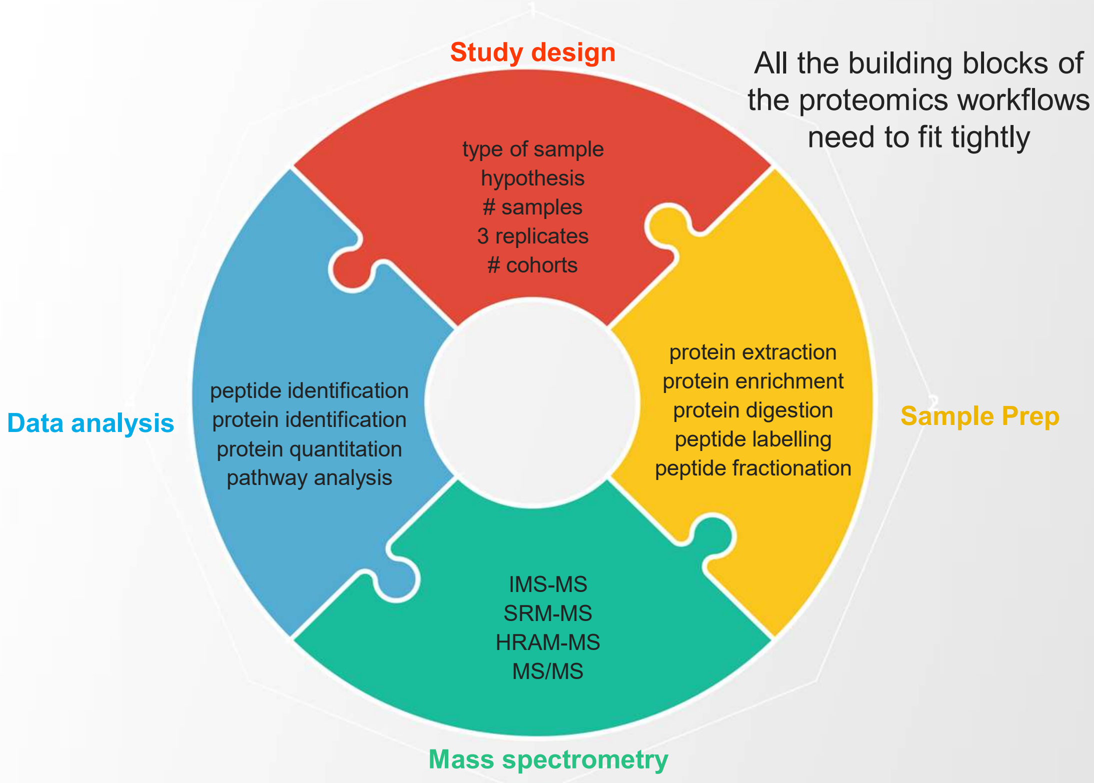
Challenges of proteomics

Protein diversity challenges the standardization and simplification of proteomics workflows

4 bases



20 amino acids



Study design

type of sample
hypothesis
samples
3 replicates
cohorts

Sample Prep

protein extraction
protein enrichment
protein digestion
peptide labelling
peptide fractionation

Mass spectrometry

IMS-MS
SRM-MS
HRAM-MS
MS/MS

Data analysis

peptide identification
protein identification
protein quantitation
pathway analysis

All the building blocks of the proteomics workflows need to fit tightly

Democratization of proteomics requires standardization and simple protocols

Proteomics analysis - Economics

Sample preparation can negatively impact the entire workflow

Complex protocols
Negatively affect the chance for success

Long waiting times
Very prone to errors

Standardization
Unpredictable/ Irreproducible results

Costly to serve
They believe bad results are tied to LCMS

Everyone is in crisis
Loses precious samples, time & money

The missing piece

A complete solution

Improving sample preparation will significantly **increase data quality and productivity** in proteomics labs while reducing the overall cost of operations.

Making every sample count

The role of Thermo Fisher Scientific to provide the entire proteomics workflow solution

Samples → Sample prep → Proteomic assays → Data analysis

10-100 µg
protein input

- Plasma
- Tissue lysates
- Purified proteins
- Cell lysates

Fill the gap

5-40 µg peptide output
+ PRTC

Thermo Scientific™ Vanquish Neo™ UHPLC system

Exceptional retention time precision

Thermo Scientific™ µPAC™ pillar array columns

Optimal performance and an unprecedented reproducibility and robustness

Thermo Scientific™ Orbitrap™ mass spectrometers

Orbitrap Exploris 240 MS

Orbitrap Exploris 480 MS

Orbitrap Eclipse Tribrid MS

Thermo Scientific™ Proteome Discoverer™ software with MSAID GmbH's CHIMERYS search engine

More peptide identifications for maximum confidence

Proteomics sample preparation

Innovation is in our DNA

Supported workflows

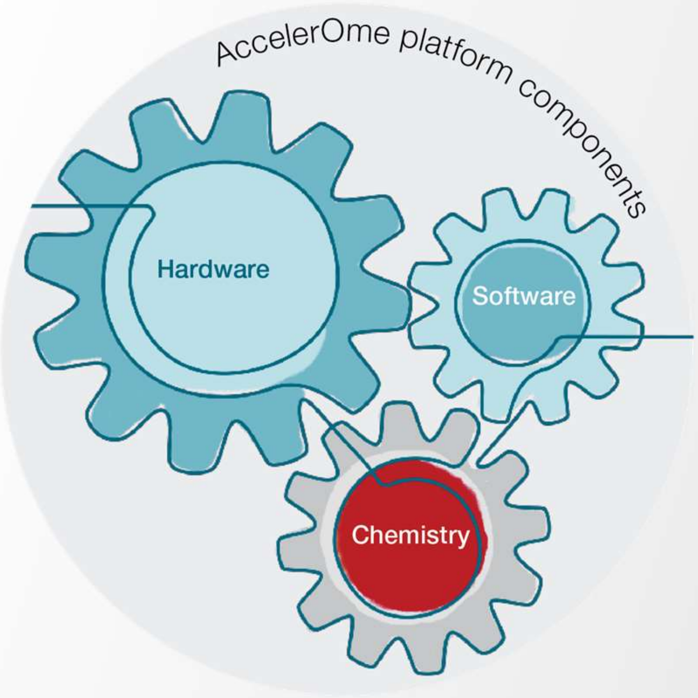
Tandem Mass Tags (TMTs) multiplexing

Label-free quantitation

Protein identification

Improvements

- Standardization
- ↓Operation costs/Time
- ↑Sample processing
- High-quality data
- Reproducibility



Thermo Scientific™ AccelerOme™ automated sample preparation platform

Robust & precise HW

Elegant built-for-purpose automated liquid handler unlike others

Factory-verified reagents

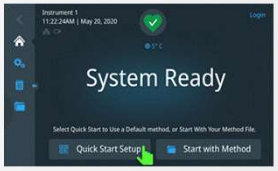
Thermo Scientific™ Label-free sample preparation kits

Thermo Scientific™ TMT11plex sample preparation kits

Thermo Scientific™ TMTpro™ 16plex sample preparation kits

Ready-to-use kits that require minimal hand-on time to set the experiments

End-to-end software



Instrument 1
11:22:24AM | May 20, 2020

Login

5° C

System Ready

Select Quick Start to Use a Default method, or Start With Your Method File.

Quick Start Setup

Start with Method

Quick Start Setup | Step 1 of 3
Select Kit Type

Select kit, or scan kit box with QR code

Label-Free 16

Label-Free 36

TMT11 100 µg (11)

Back

Robust and intuitive software supports scientists in their journeys from the experiment design through data analysis

Reduced to the simplicity of an automatic espresso machine

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Sample information transfer from start to finish

The experimental design and other metrics such peptide amount information are carried out along the process to be consumed for improved results and reduced operator errors.

Support experimental design

Extract proteins

(Cells, tissue, plasma)

Load sample & kit components

Reduction

Alkylation

Trypsin/LysC digestion

Peptide pooling & clean-up

Peptide concentration measurement

Dry and reconstitute samples

Analyze by LC-MS

Analyze data and results

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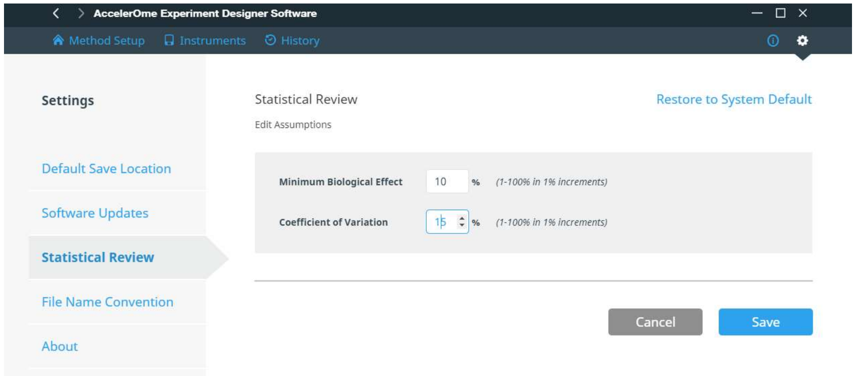
Unique experimental design software

1. Establish your statistical assumptions



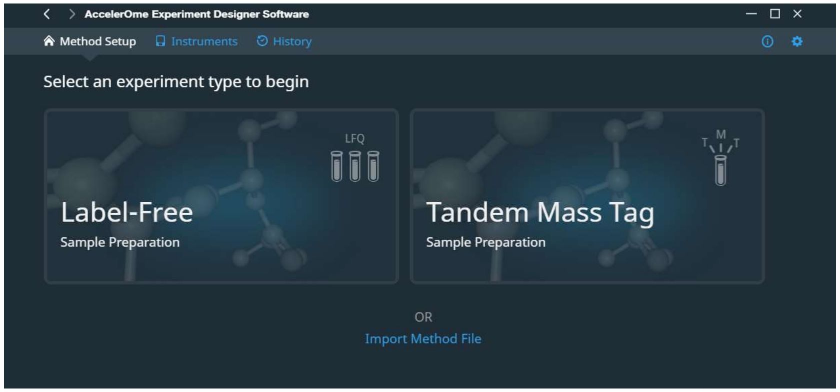
Statistical Review

Edit Assumptions

Minimum Biological Effect 10 % *(1-100% in 1% increments)*

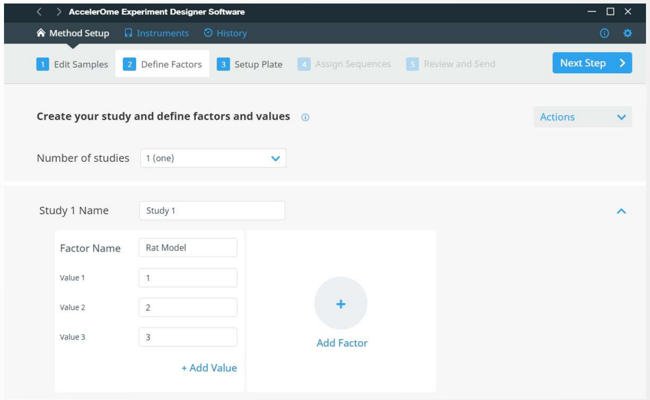
Coefficient of Variation 15 % *(1-100% in 1% increments)*

2. Select your quantitative proteomics workflow



Select an experiment type to begin

3. Provide factors and values



Create your study and define factors and values

Number of studies: 1 (one)

Study 1 Name: Study 1

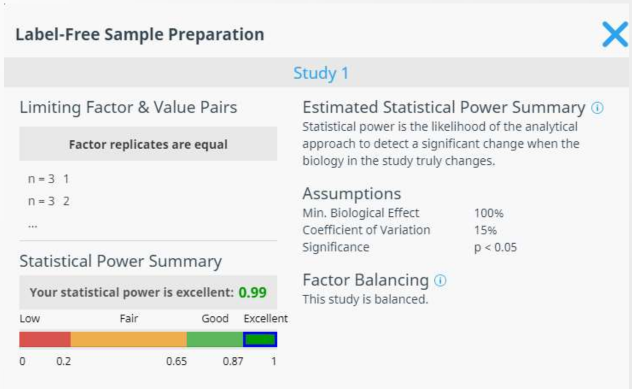
Factor Name: Rat Model

Value 1: 1

Value 2: 2

Value 3: 3

4. Review your experiment



Label-Free Sample Preparation

Study 1

Limiting Factor & Value Pairs

Factor replicates are equal

n = 3 1

n = 3 2

...

Statistical Power Summary

Your statistical power is excellent: 0.99

Estimated Statistical Power Summary

Statistical power is the likelihood of the analytical approach to detect a significant change when the biology in the study truly changes.

Assumptions

Min. Biological Effect	100%
Coefficient of Variation	15%
Significance	$p < 0.05$

Factor Balancing

This study is balanced.

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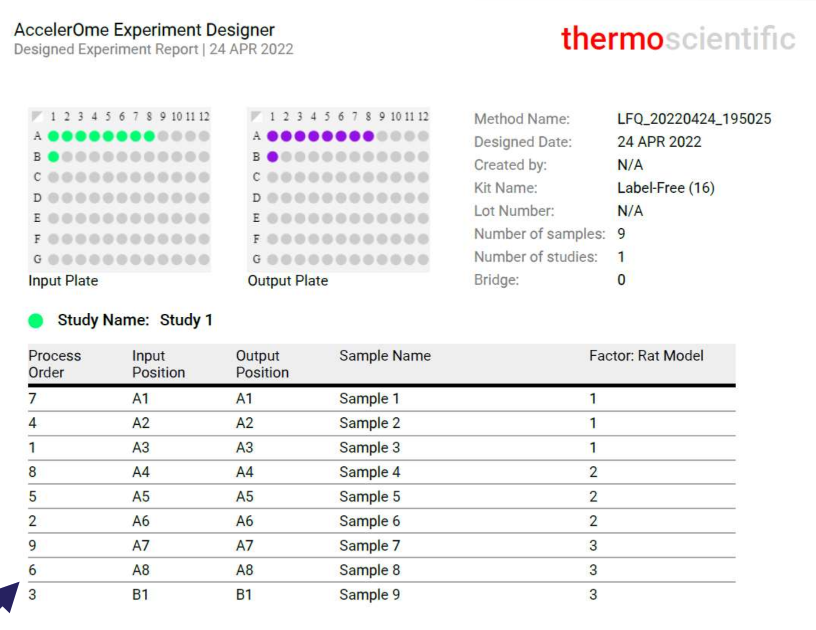
Unique experimental software

Randomization import/export to workflow

Sample preparation order is randomized to avoid any analytical bias



AccelerOme Experiment Designer
Designed Experiment Report | 24 APR 2022

Input Plate

Output Plate

Method Name:	LFQ_20220424_195025
Designed Date:	24 APR 2022
Created by:	N/A
Kit Name:	Label-Free (16)
Lot Number:	N/A
Number of samples:	9
Number of studies:	1
Bridge:	0

● **Study Name: Study 1**

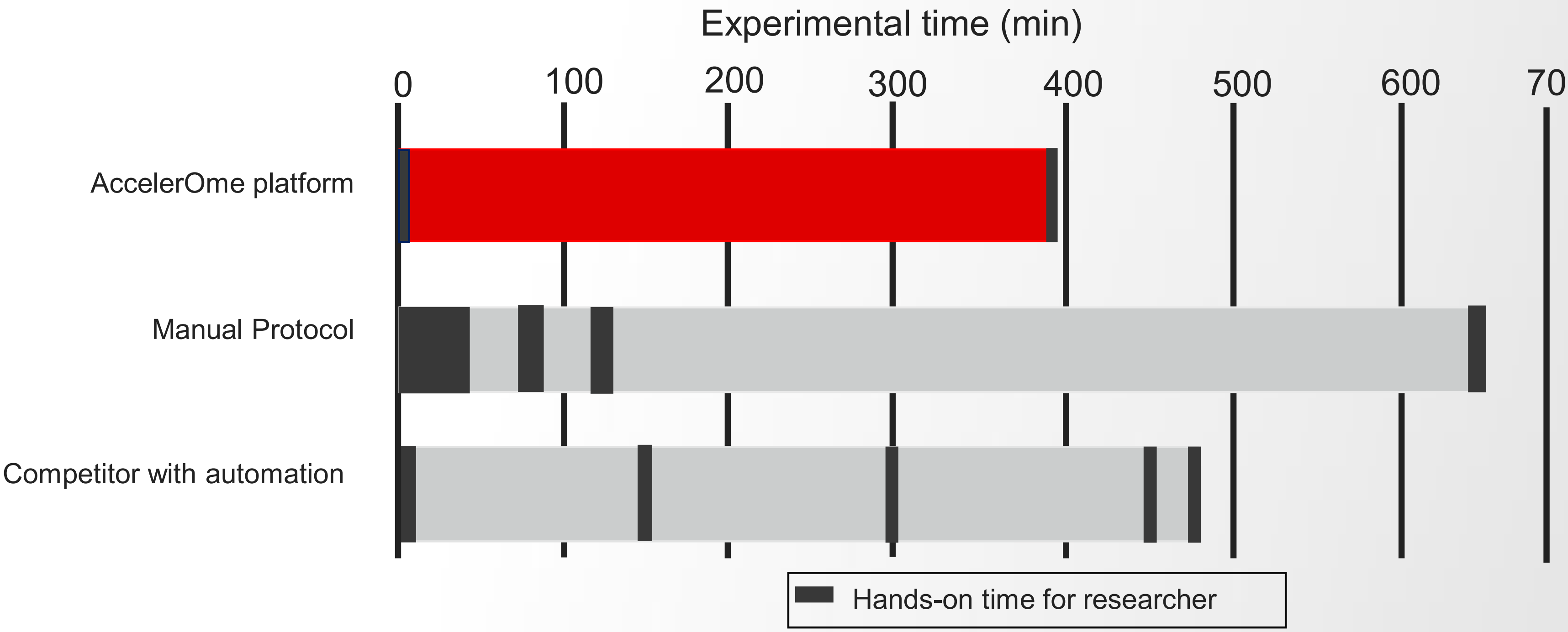
Process Order	Input Position	Output Position	Sample Name	Factor: Rat Model
7	A1	A1	Sample 1	1
4	A2	A2	Sample 2	1
1	A3	A3	Sample 3	1
8	A4	A4	Sample 4	2
5	A5	A5	Sample 5	2
2	A6	A6	Sample 6	2
9	A7	A7	Sample 7	3
6	A8	A8	Sample 8	3
3	B1	B1	Sample 9	3

Thermo Scientific™ LC system with Thermo Scientific™ Orbitrap™ mass spectrometer

Designs experiment to deliver rigorous results that matter

Minimal hands-on time

Time and effort needed to prepare 36 high quality sample



Avoid interruptions and spend time on more valuable tasks

Software wizard for operational simplicity

- Guides user through step-by-step experimental set-up
- Provides final deck layout for reagents and samples after experimental design step
- No complex programming of methods
- Saves sample information (including study factors) for downstream use

Run Complete

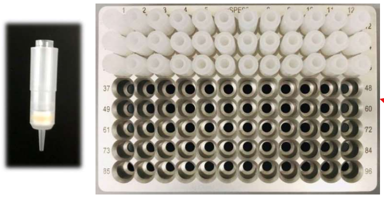
Remove All Instrument Content

1. Remove and save sample output plate from position A.
2. Remove and discard reagents, µSPE rack, liquid waste, and wash buffers.
3. Close door.

Democratizes sample preparation for levels of users

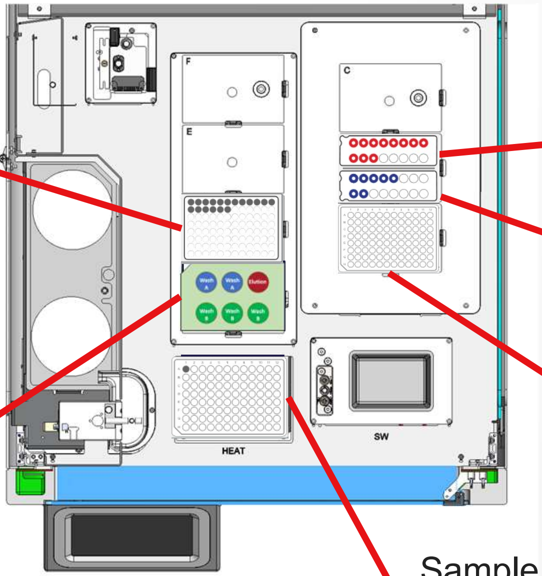
Reagent Loading onto AccelerOme System

For ease-of-use reagent trays are directly inserted into the instrument slots



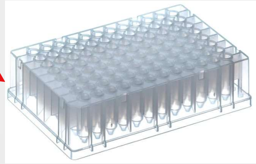
μSPE Cartridge Tray

Peptide Clean-up Buffer Tray

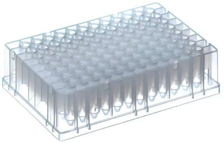


TMT Labeling Reagent Tray

Sample Prep Reagent Tray



Sample Output Plate

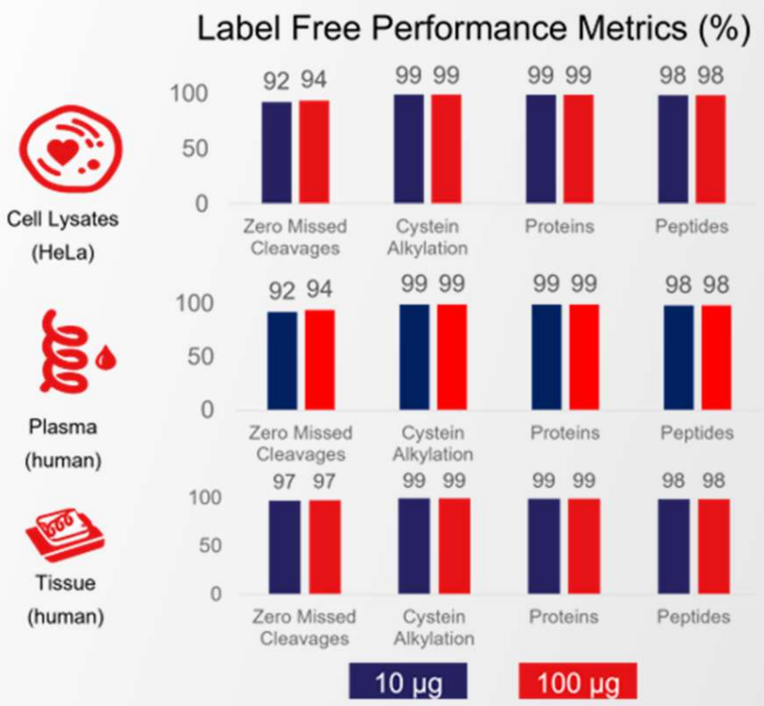


Sample Input Plate

Factory-verified kits and reagents

High-quality samples for downstream LC-MS analysis

- Optimized chemistries minimize artifacts that can disrupt and have no bearing on final results
- Maximizing sample quality increases confidence for downstream analysis
- Convenience without sample losses
- Robust sample preparation metrics
- Applicable to many different sample types



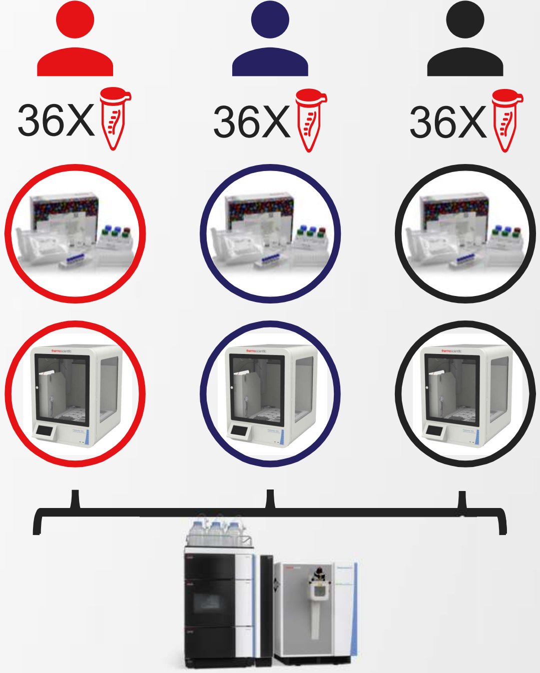
Sample versatility

High-quality samples, no matter the source

Requirements that matter

Sample types	Cell lysates, plasma, tissue, purified proteins
Total protein amount	10 µg – 100 µg
0 Missed cleavage	≥90%
2 Missed cleavage	≤2%
Methionine oxidation %	≤10%
Cysteine alkylation %	≥96 %
Off-target alkylation %	≤5%
N-Deamidation %	≤10%
Optional TMT labeling	TMT 11, TMTpro 16
Labeling efficiency %	>95%

Robust testing like you would do

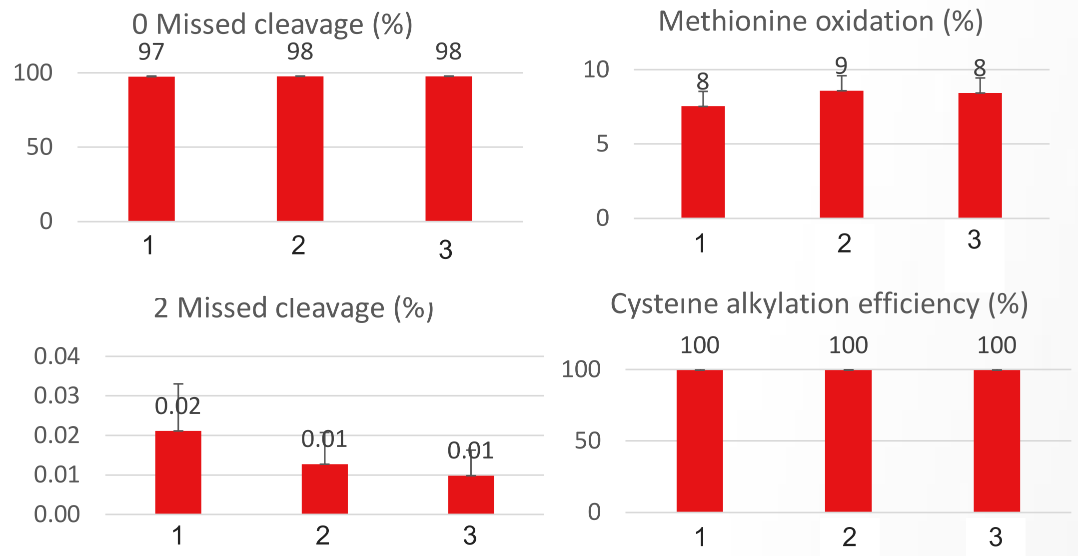


Unmatched AccelerOme platform performance with HeLa cells

Excellent reproducibility across platforms and users

3 Different users on separate AccelerOme platforms and separate label-free kit for 36 samples

Sample preparation quality metrics



LC-MS metrics

Exp. #	AccelerOme platform	User #	# of samples	Average peptide ID	%RSD
1	AccelerOme platform 1	1	36	18229	4.31
2	AccelerOme platform 2	2	36	15408	2.17
3	AccelerOme platform 3	3	36	15748	2.50

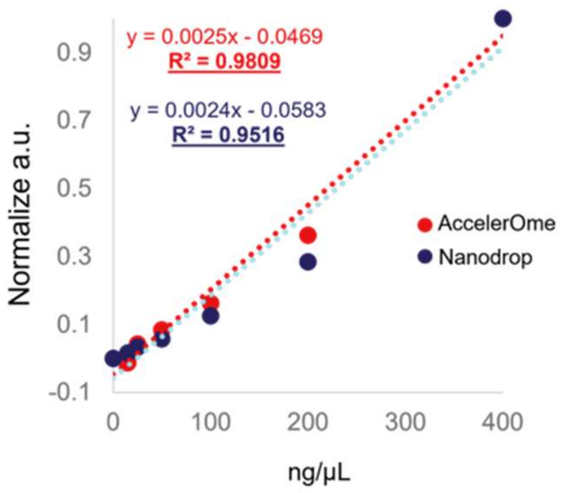
Exp. #	AccelerOme platform #	User #	# of samples	Average peptide ID	%RSD
1, 2, 3	1, 3, 4	1, 2, 3	108	16461	8.34

High sample quality maintained with different users, platforms, and kits

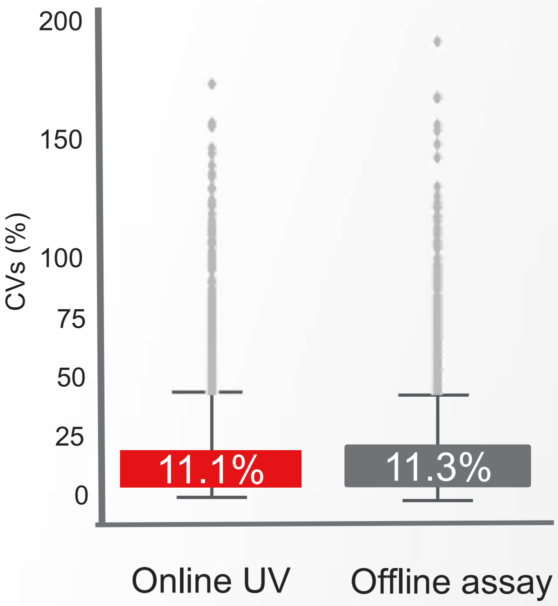
Online peptide quantitation

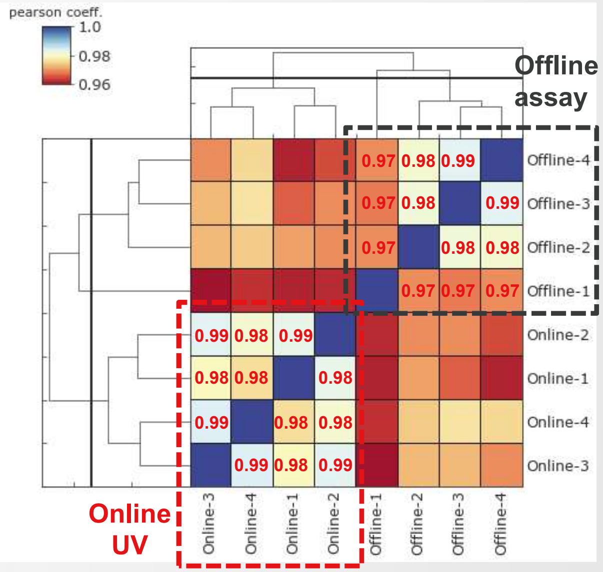
Benchmark with offline Thermo Scientific™ NanoDrop™ system

Performance benchmark against technology leader



Precision benchmark on plasma prep using LCMS to evaluate biases between online and offline peptide normalization assays





Faster path to high-quality proteomics LC-MS analysis

1. Medium throughput proteomics study

Analysis of stored red blood cells from 18 non-domesticated cat species

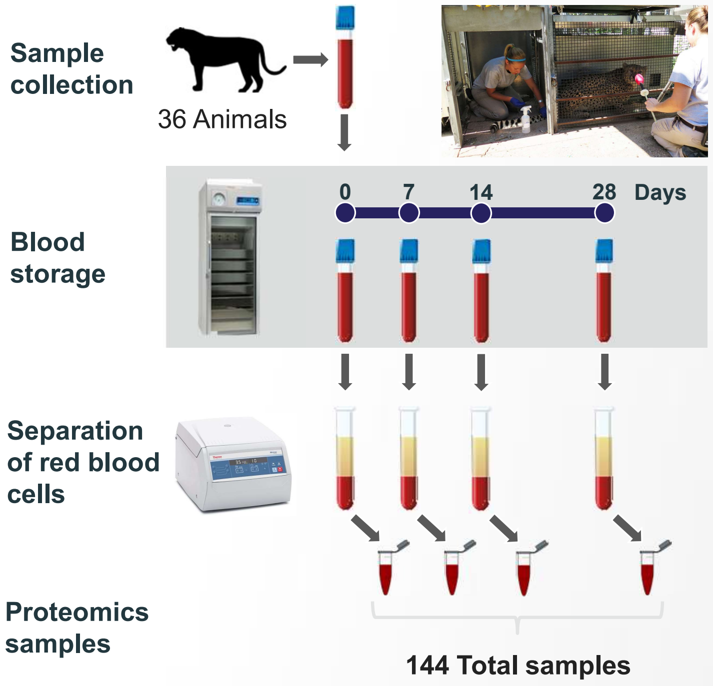
- Blood transfusions are a life-saving treatment
- Little known about different blood types in non-domesticated cat species
- Zoos typically have a few individuals of each species so there is a need to either transfuse blood from one species to another or store blood for prolonged periods of time
- Delivers turn-key solution for high-quality samples

144 samples from 18 species

Dr. Lily Parkinson
Milwaukee County Zoo

Study design

Analysis of stored red blood cells from 18 non-domesticated cat species



Sample collection

36 Animals

Blood storage

0 7 14 28 Days

Separation of red blood cells

Proteomics samples

144 Total samples

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Label-free quantitation

AccelerOme platform - Single platform for flexible workflows and predictable timelines

Materials & methods

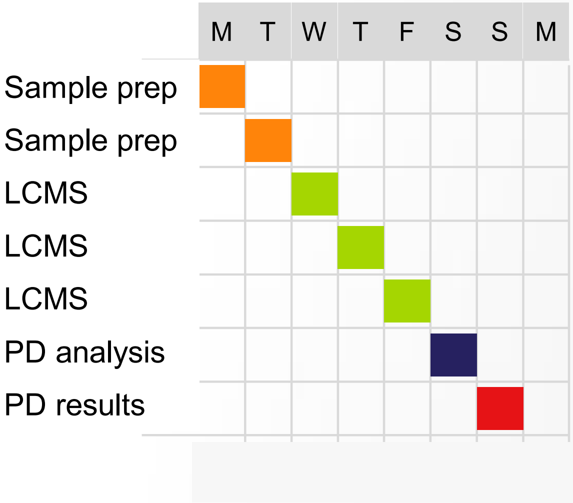
Instrumentation & consumables

- 1 AccelerOme platform
- 4 LFQ 36 kits
- 1 Thermo Scientific™ UltiMate™ 3000 RSLCnano system
- 1 Orbitrap Exploris 480 MS
- 1 Thermo Scientific™ EASY-Spray™ HPLC column 150 µm x 150 mm C18 column

Methodology

- 26-minute method (55 sample/day)
- 2 FAIMS CV settings
- Proteome Discoverer 3.0 software

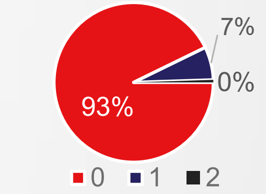
Timeline

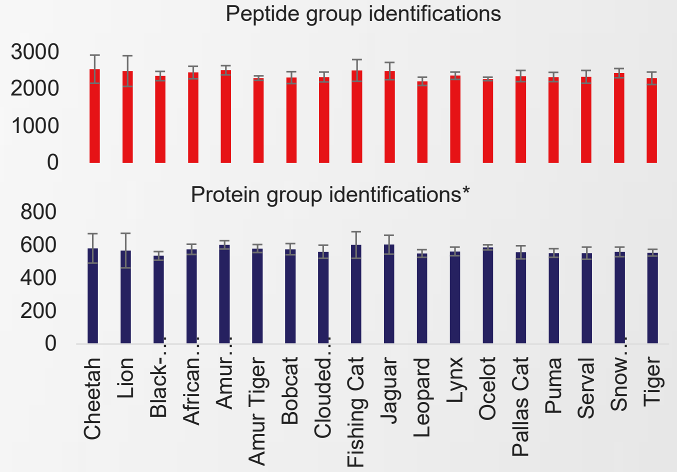


	M	T	W	T	F	S	S	M
Sample prep	🟧							
Sample prep		🟧						
LCMS			🟩					
LCMS				🟩				
LCMS					🟩			
PD analysis						🟦		
PD results							🟥	

Preliminary data

Digestion efficiency




Peptide group identifications

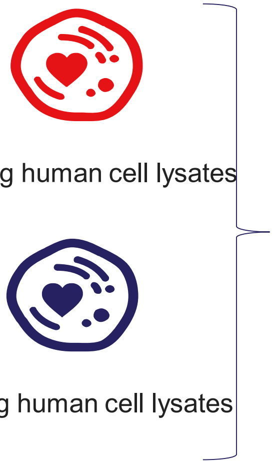
Protein group identifications*

Enabling medium throughput proteomics study >50 samples per day

TMT multiplexing: Hands-off labeling

AccelerOme platform - Single system for flexible workflows

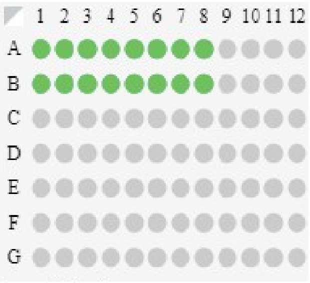
- Experimental design
- Cell lysis
- Protein reduction & alkylation
- TMT labeling
- Pooling
- Clean-up
- Peptide UV assay
- Aliquotting online



8 X 30 µg human cell lysates

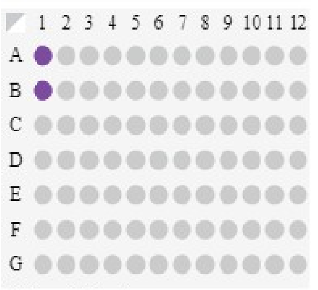
8 X 60 µg human cell lysates

X 2



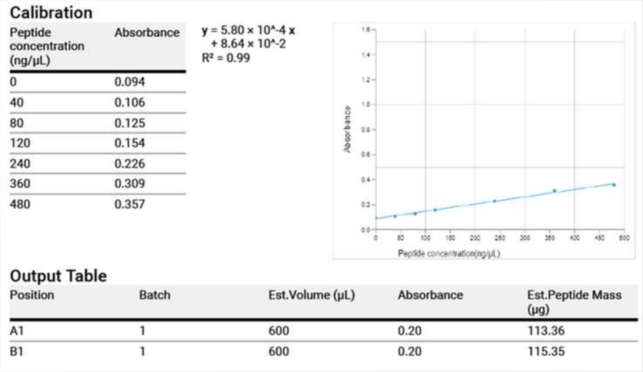
	1	2	3	4	5	6	7	8	9	10	11	12
A	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	⚪	⚪	⚪	⚪
B	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	⚪	⚪	⚪	⚪
C	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
D	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
E	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
F	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
G	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪

Input Plate



	1	2	3	4	5	6	7	8	9	10	11	12
A	🟣	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
B	🟣	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
C	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
D	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
E	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
F	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪
G	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪	⚪

Output Plate



Calibration

Peptide concentration (ng/µL)	Absorbance
0	0.094
40	0.106
80	0.125
120	0.154
240	0.226
360	0.309
480	0.357

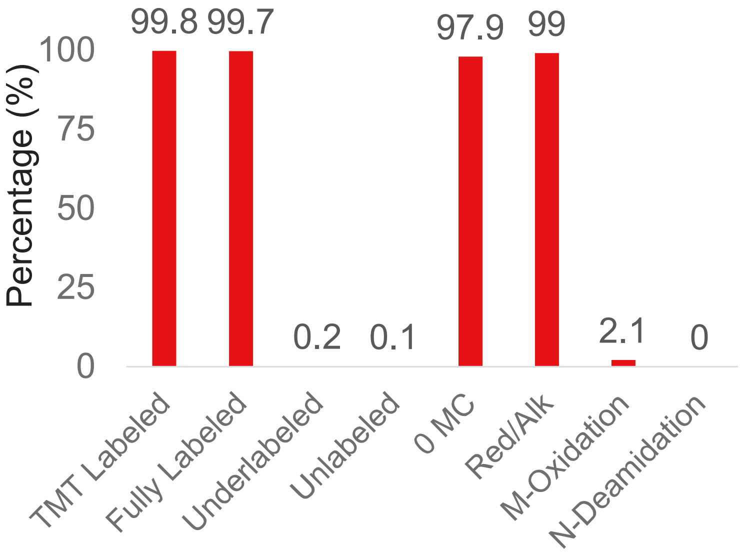
y = 5.80 × 10^-4 x + 8.64 × 10^-2
R² = 0.99

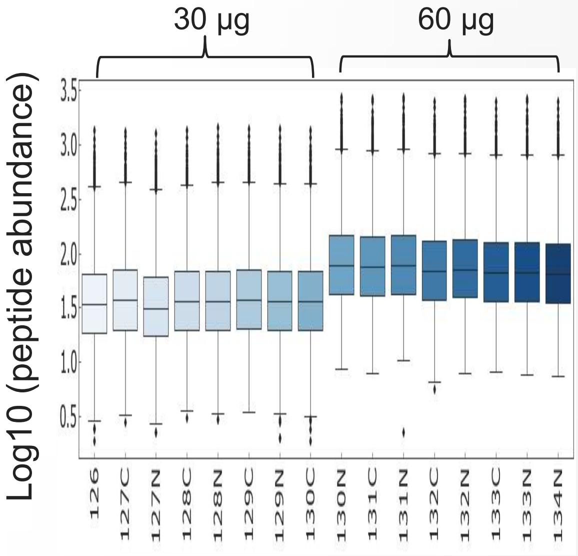
Output Table

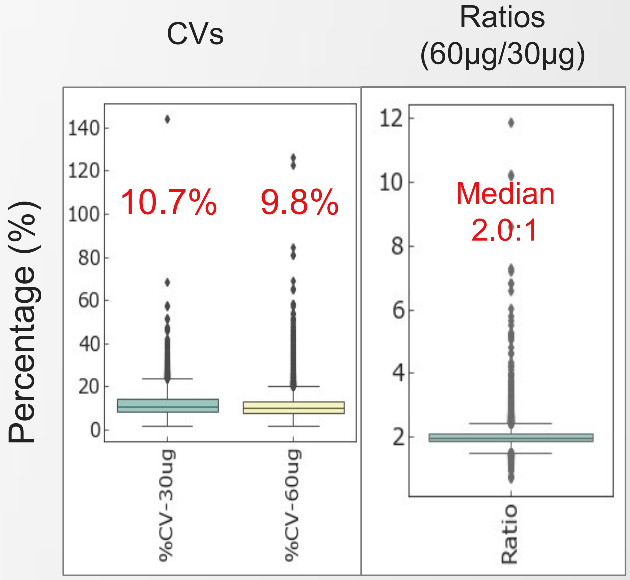
Position	Batch	Est.Volume (µL)	Absorbance	Est.Peptide Mass (µg)
A1	1	600	0.20	113.36
B1	1	600	0.20	115.35

TMT multiplexing: Hands-off labeling

Excellent quality metrics and quantitation precision and accuracy for human cells







Labeling efficiency

MS sample quality

Quantitation without normalization

Integrated Orbitrap MS proteomics workflow

minimal user intervention

Experimental design

Protein extraction and lysis

Loading the kit components

Automated sample preparation

Dry and reconstitute the samples

LC-MS analysis

Data processing

Experimental design at your desk

Lysis using lysis buffer for most sample types

Load the samples and ready racked kit components

Reduction

Alkylation

Digestion

Peptide pooling and clean-up

Peptide concentration measurement

Dry and reconstitute to the concentration desired

LC-MS data acquisition with Orbitrap mass spectrometer

Identification and quantitation using Proteome Discoverer software with CHIMERYS

Sample information such as study factors etc.

→

Kits and sample information carried through to deck layout

→

Sample information carried through to Xcalibur software that sets up sequence

→

Sample information carried through to Proteome Discoverer software for data analysis

Kristan.Bahten@thermofisher.com | 5 June 2022

Optimized to fit into the Orbitrap MS ecosystem

Unique sample information transfer and workflow integration from start to finish

Sample prep

Proteomic analysis

Data analysis

Thermo Scientific™ AccelerOme™ platform

Study design and sample information in Experiment Designer software

Sample & kit layout for ease-of-use

Thermo Scientific™ Orbitrap™ mass spectrometers

Sample information transfers to LC-MS run via automated generation of the sequence list

Thermo Scientific™ Proteome Discoverer™ software with CHIMERYS

Sample information plus study factor/metadata transferred to data analysis software and reporting

Thank you

Learn more at thermofisher.com/AccelerOme