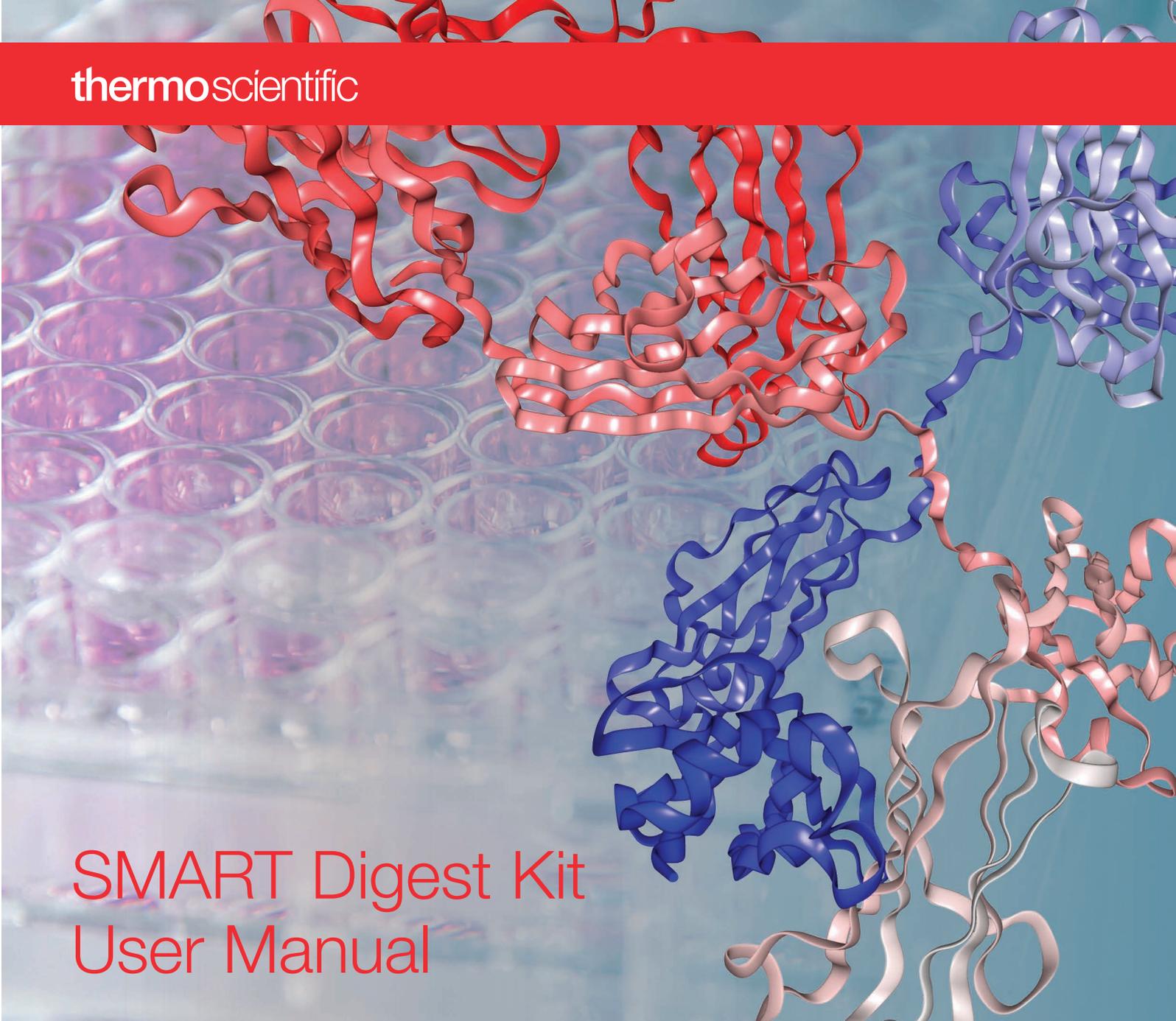


thermoscientific



SMART Digest Kit User Manual

Version 2

XX21543 Revision A • August 2016

ThermoFisher
SCIENTIFIC

SMART Digest Kit

Delivering simple, fast and reproducible digestion of proteins

Thermo Scientific™ SMART Digest™ kits are designed for biopharmaceutical and proteomic applications which require simple, fast and highly reproducible digestion of proteins. This is achieved through the use of optimized, heat stable, enzymes.

The SMART Digest kits are available in a number of different formats to meet your workflow requirements.

Part Number	Description	Contents
Non-Magnetic Options		
60109-101	SMART Digest Kit Trypsin	<ul style="list-style-type: none">• 96 SMART Digest tubes• SMART Digest buffer• Collection plate
60109-104	SMART Digest Kit Chymotrypsin	
60109-107	SMART Digest Kit Proteinase K	
60109-102	SMART Digest Kit Trypsin	<ul style="list-style-type: none">• 96 SMART Digest tubes• SMART Digest buffer• Collection plate• Filter plate
60109-105	SMART Digest Kit Chymotrypsin	
60109-108	SMART Digest Kit Proteinase K	
60109-103	SMART Digest Kit Trypsin	
60109-106	SMART Digest Kit Chymotrypsin	<ul style="list-style-type: none">• 96 SMART Digest tubes• SMART Digest buffer• Collection plate• Thermo Scientific™ SOLAμ™ SPE plate
60109-109	SMART Digest Kit Proteinase K	
Non-Magnetic Bulk Options		
60109-101-B	SMART Digest Kit Trypsin	<ul style="list-style-type: none">• SMART Digest resin in bulk format• SMART Digest buffer• Collection plate
60109-104-B	SMART Digest Kit Chymotrypsin	
60109-107-B	SMART Digest Kit Proteinase K	
60109-102-B	SMART Digest Kit Trypsin	<ul style="list-style-type: none">• SMART Digest resin in bulk format• SMART Digest buffer• Collection plate• Filter plate
60109-105-B	SMART Digest Kit Chymotrypsin	
60109-108-B	SMART Digest Kit Proteinase K	
60109-103-B	SMART Digest Kit Trypsin	
60109-106-B	SMART Digest Kit Chymotrypsin	<ul style="list-style-type: none">• SMART Digest resin in bulk format• SMART Digest buffer• Collection plate• SOLAμ SPE plate
60109-109-B	SMART Digest Kit Proteinase K	

Magnetic Bulk Options

60109-101-MB	SMART Digest Kit Trypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-104-MB	SMART Digest Kit Chymotrypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-107-MB	SMART Digest Kit Proteinase K	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate • Filter plate
60109-102-MB	SMART Digest Kit Trypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-105-MB	SMART Digest Kit Chymotrypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-108-MB	SMART Digest Kit Proteinase K	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate • SOLAμ SPE plate
60109-103-MB	SMART Digest Kit Trypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-106-MB	SMART Digest Kit Chymotrypsin	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate
60109-109-MB	SMART Digest Kit Proteinase K	<ul style="list-style-type: none"> • SMART Digest resin in bulk format • SMART Digest buffer • Collection plate • SOLAμ SPE plate

Soluble Enzyme Option (For use with complex sample sets)

60113-101	SMART Digest Kit Soluble Trypsin	<ul style="list-style-type: none"> • SMART Digest soluble trypsin • SMART Digest buffer • Collection plate
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Product Storage

It is important to store the components of the SMART Digest kits correctly to ensure the product lifetime and performance.

- SMART Digest tubes and resin materials store at -20 °C.
- SMART Digest buffer store at 4 °C.
- All other components store at room temperature.

The SMART Digest kits ship in cold conditions. Each kit contains a WarmMark^{®2} Temperature Indicator. This indicator tracks how long the kit has been at, or above, 38 °C by irreversibly turning from white to blue.

If on opening the kit the temperature sensor indicates that the kit has been exposed to 38 °C for more than 2 hours please contact Technical Support: www.thermofisher.com/chromexpert to help determine the functionality of the SMART Digest kit.

WarmMark^{®2} Time/Temperature Indicator



Materials recommended, but not provided:

- Heater/shaker equipped with heated block and heated lid, capable of uniformly heating samples to 70 °C.
- Thermo Scientific™ DynaMag™ -96 Side Magnet block for use with magnetic bead versions of kit (P/N 12331D).
- The Thermo Scientific™ KingFisher™ Duo Prime, or KingFisher Flex Purification Systems (P/N 5400110 and P/N 5400630 respectively) for use with magnetic bead versions of the kit (high throughput options).

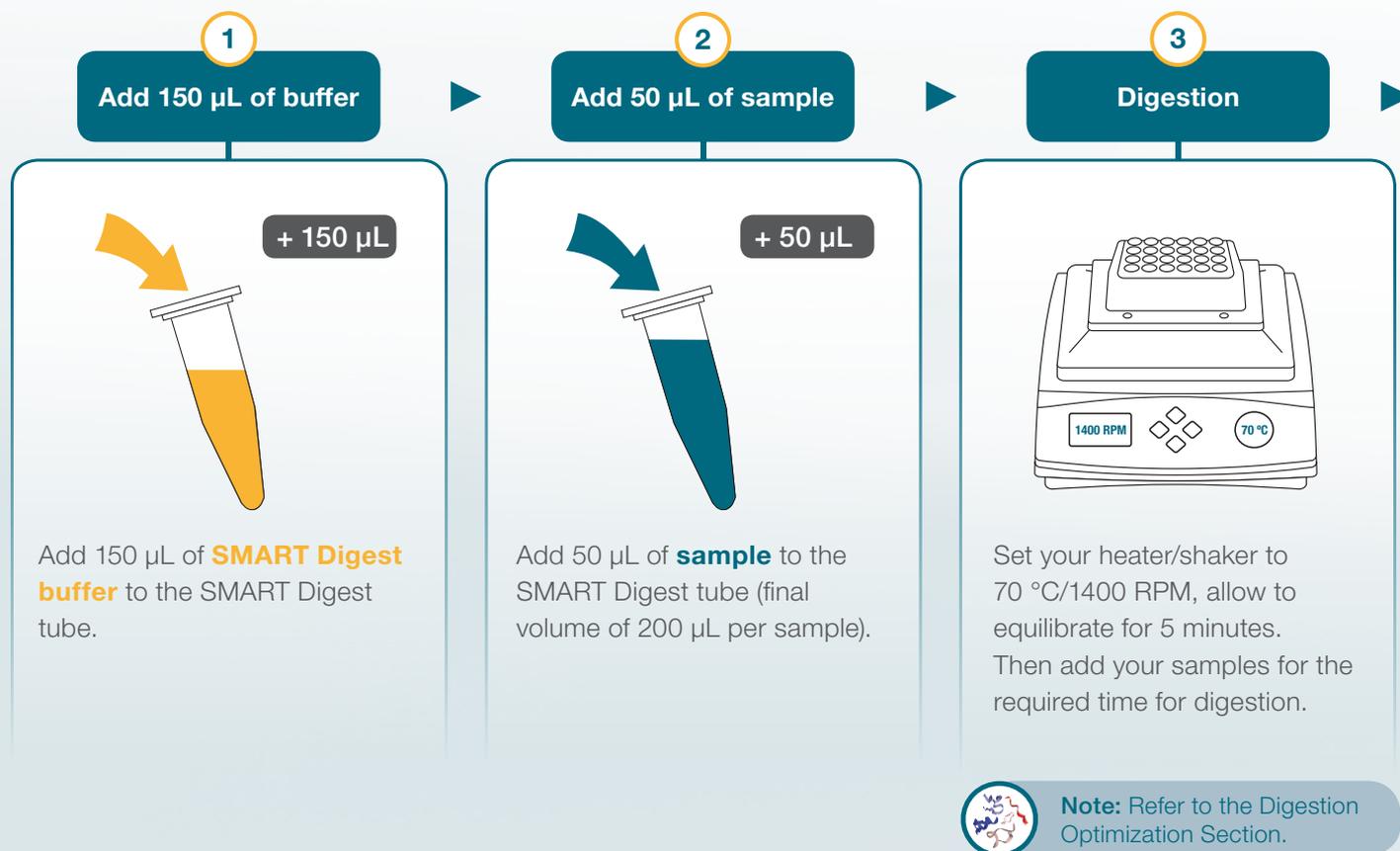
For use with filter plate or SOLA μ SPE plate operation:

- Thermo Scientific™ HyperSep-96 Well Plate Vacuum Manifold (P/N 60103-351).
- The Thermo Scientific™ Vacuum Pump NA option (P/N 60104-243) or EU option (P/N 60104-241).

Recommended column for peptide quantification:

Thermo Scientific™ Acclaim™ VANQUISH™, C18, 2.2 μ m Analytical (2.1 × 250 mm, P/N 074812-V).

How to use your SMART Digest kit



Questions Answers

Q: Why is the temperature set to 70 °C?

This has been shown to be the optimum temperature for enzyme activity and the unfolding of proteins. Higher or lower temperatures may reduce the efficiency of the digestion.

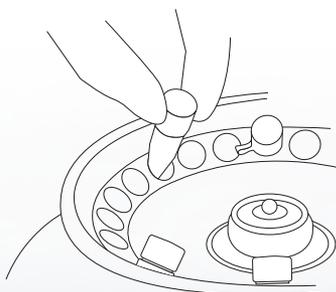
Q: What concentration of protein can I add to each SMART Digest tube?

You can add 200 pg to 3.5 mg of total protein to each SMART Digest tube (protein/sample dependent).

Q: Do I have to reduce and alkylate my protein?

The SMART Digest kits are thermally stable. When operated at high temperatures denaturation and digestion occur simultaneously. Therefore, for many quantitative workflows, there is no need to perform the additional steps of denaturation, reduction and alkylation. However, during this process many disulfide bonds will remain intact. As a result, for characterization workflows where maximum sequence coverage is required, it is recommended that you perform reduction and alkylation after digestion.

4

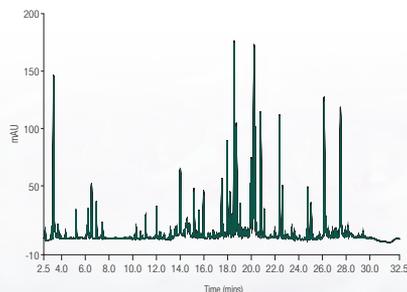
Cleanup

Remove the SMART Digest tube from the heater/shaker and perform one of the sample cleanup methods based on the complexity of your starting matrix.



Note: Refer to the Post Digestion Process.

5

Analyze

Analyze sample.

Q: My protein is very difficult to unfold so I need to use urea. What concentration of urea can I use?

The SMART Digest kit uses heat to unfold the protein. If urea is required then it is recommended to dilute the sample to 0.5 M or less of urea prior to transferring to the SMART Digest tube.

Q: What if I have less than 50 μ L of sample?

If your sample is less than 50 μ L adjust to 50 μ L with ultrapure water.

Q: Does digestion using the SMART Digest kit at high temperatures result in an increase in post-translational modifications?

In comparison to in-solution digests a comparable number of PTMs have been observed when screening for deamidation, amidation, methylation and oxidation. No modifications to existing PTMs, such as phosphorylated sites, have been observed.

Digestion Optimization

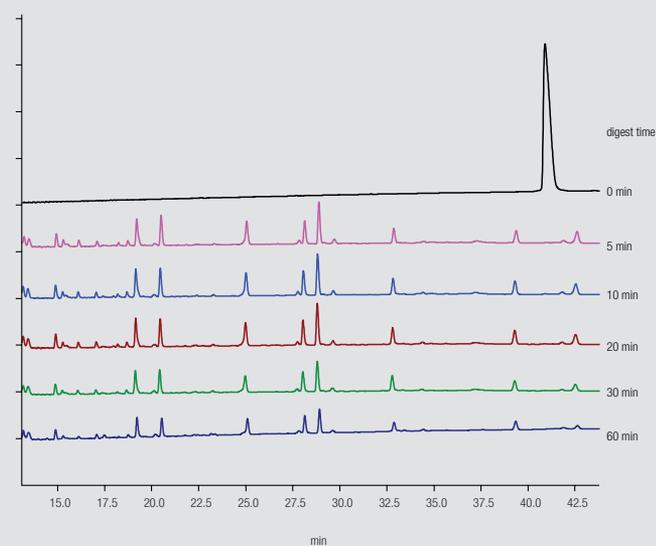


Optimize the time for your assay

- Prepare eight identical samples as described previously using a relatively high, known concentration of native analyte in the matrix of operation.
- Digest the samples according to the Protein Digestion Procedure and periodically remove one of the samples from the well or tube (e.g. every 5 to 15 minutes).
- Perform the appropriate low, medium or high complexity post digestion process.
- Analyze the samples to determine the extent of digestion (see chromatogram and table below).
- Disappearance of the intact protein peak and stabilization of peptide peak intensity and peak ratios indicates a complete digestion. Once stable, the corresponding digest time can be used for subsequent analyses. By removing consecutive samples and monitoring these features the optimum digestion time can be determined.
- For carbonic anhydrase full digestion is complete in 5 minutes.

Carbonic Anhydrase, 29 KDa

Time course experiment for digestion optimization



Typical Digestion Times

Protein	Digest Time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 μ L plasma*	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

200 μ L protein solution (100 μ g/mL) at 70 $^{\circ}$ C
*IgG in plasma (17.5 mg/mL total protein) at 70 $^{\circ}$ C

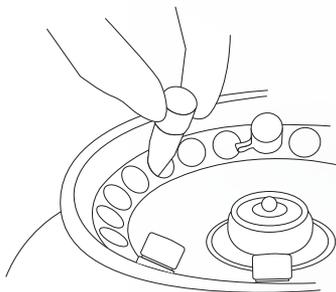
Note: DO NOT reduce and alkylate samples prior to digestion. Chemicals used for denaturation, reduction and alkylation negatively impact the activity of the protease. If the peptides of interest require reduction and/or alkylation prior to analysis it is recommended that these steps be performed post digestion.

Post Digestion Process



Choose one of the methods below based on your sample complexity and analysis requirements

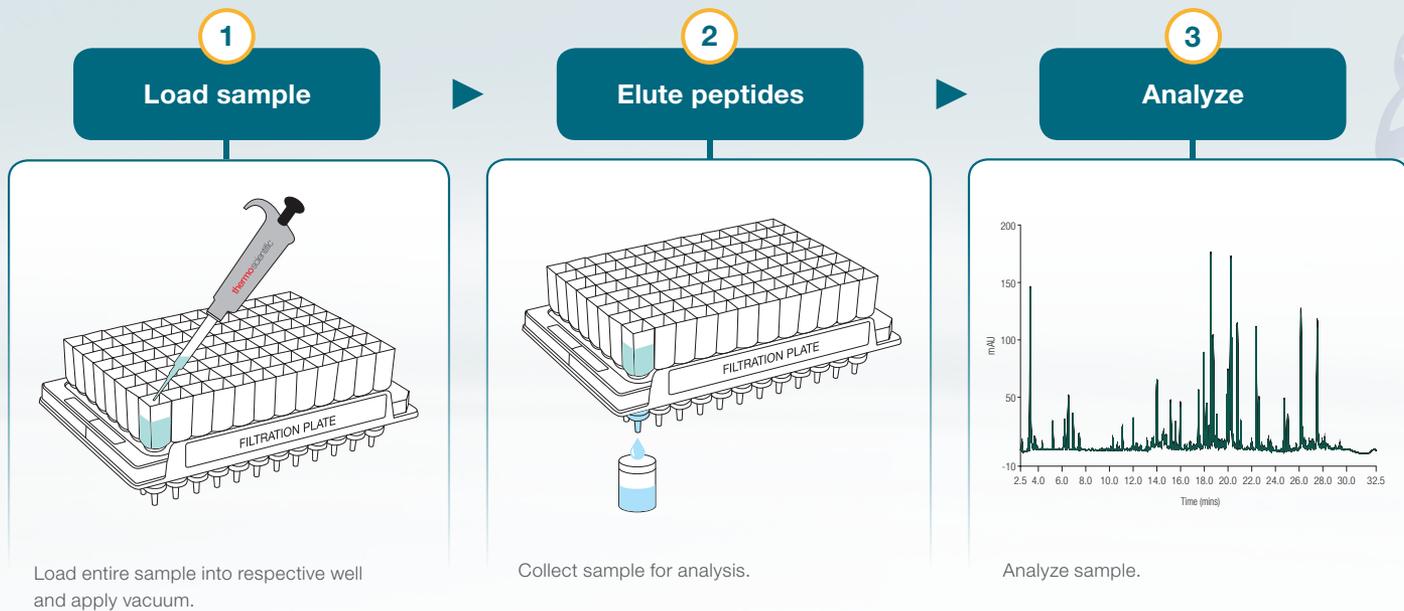
Low complexity matrix: Centrifugation



- Centrifuge sample using appropriate device.
- Remove sample from the SMART Digest tube using a pipette, making sure not to remove beads.
- Transfer supernatant samples to collection plate, or appropriate sampling holder for analysis.

Medium complexity: Filtration

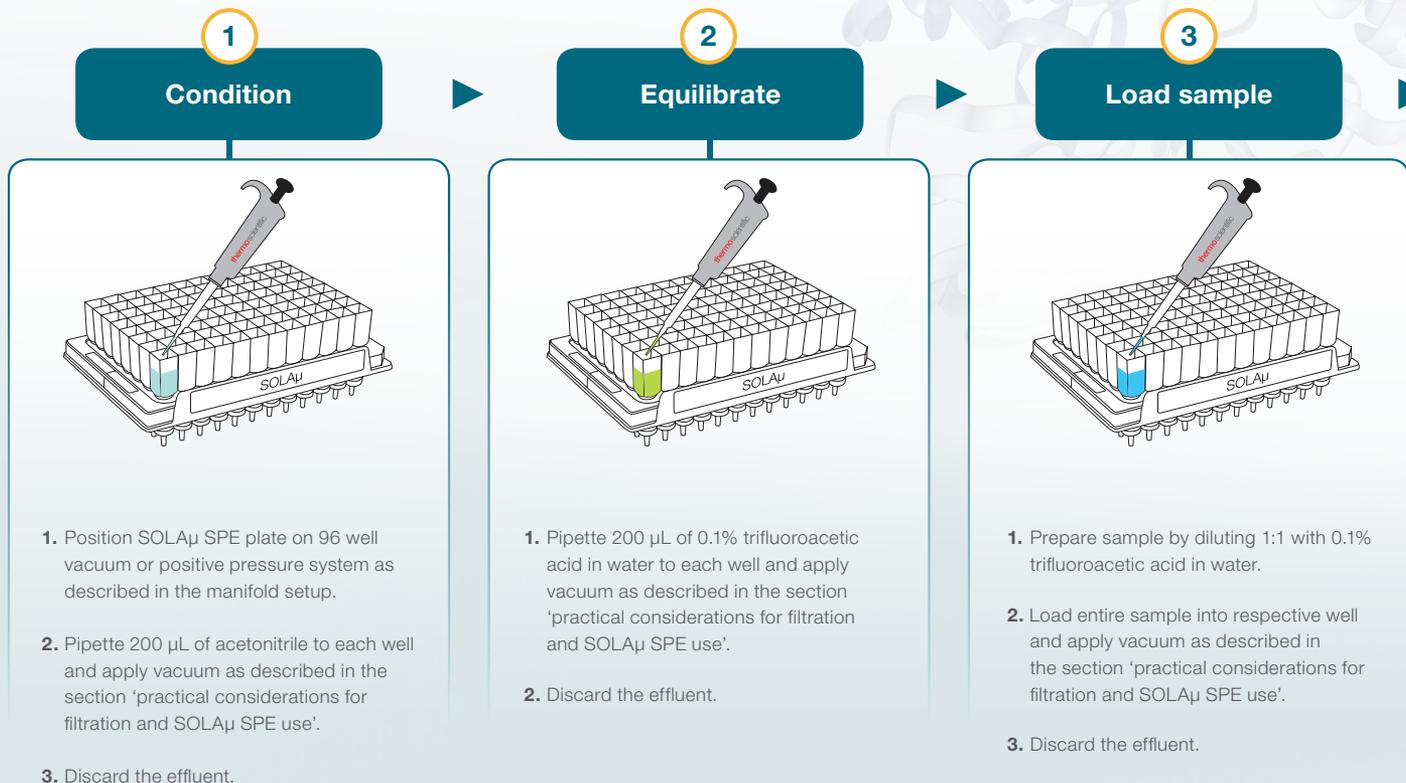
Filtration cleanup method



Note: For correct manifold setup and plate alignment, see overpage.

High complexity (e.g. plasma): SOLA μ SPE Plates

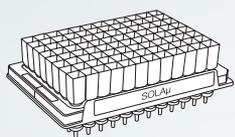
SOLA μ SPE cleanup method



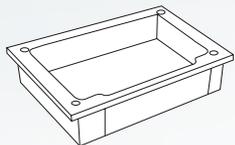
Manifold setup and plate alignment



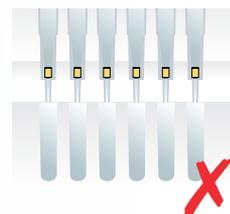
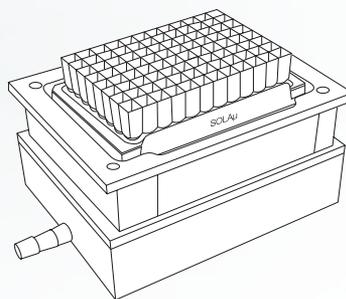
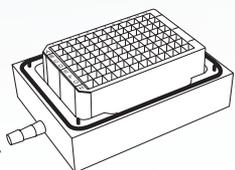
Filtration plate OR
SOLA μ SPE plate



Vacuum manifold top



96-well collection plate
in the vacuum manifold
base

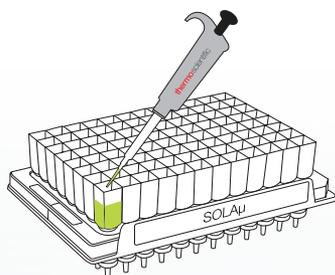


Points to consider

- No gap between filter/SOLA μ plate and collection plate.
- When under vacuum filter/SOLA μ plate will be held tightly in position.

4

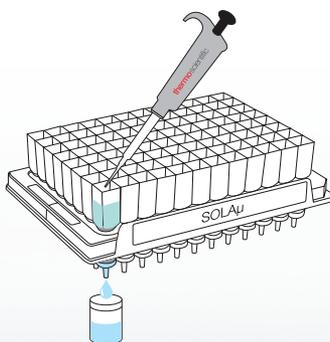
Wash



1. Pipette 500 μL of 0.1% trifluoroacetic acid in water to each well and apply vacuum as described in the section 'practical considerations for filtration and SOLA μ SPE use'.
2. Discard the effluent.

5

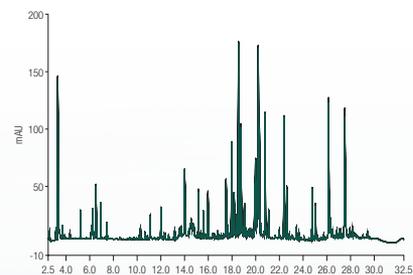
Elute peptides



1. Pipette 25 μL of 70% acetonitrile in water to respective wells as described in the section 'practical considerations for filtration and SOLA μ SPE use'.
2. Repeat this process until a total volume of 50 μL has passed through the plate.
3. **Collect sample** in appropriate vessel for analysis.

6

Analyze



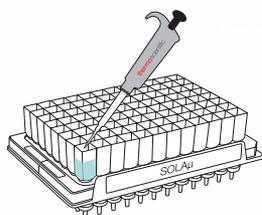
1. To your sample add 50 μL of 0.1% formic acid and mix prior to analysis.

Practical considerations for the use of filtration and SOLA μ SPE plates

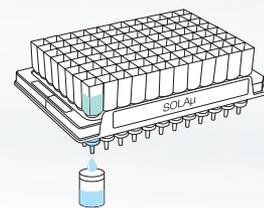
1. Using an accurate pipette, aspirate the specified volume of either solvent/reagent, or sample.



2. Dispense solvent/reagent or sample into a unique 'well' of the filtration or SOLA μ SPE plate. Up to 96 wells can be used simultaneously.



3. Apply a gentle vacuum and increase the pressure until the liquid begins to flow through the filtration, or SOLA μ SPE plate.



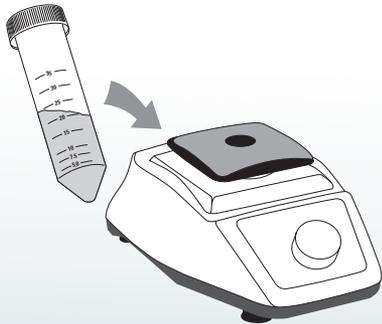
4. The collected eluent is then kept for analysis.

Note: The effluent from each load/wash step may also be collected and analyzed if method optimization is required.

How to use the SMART Digest bulk or magnetic kit

1

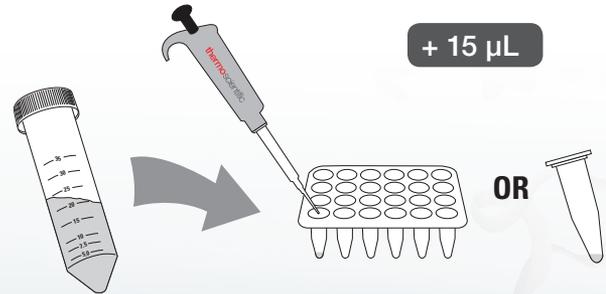
Re-suspend



Thoroughly vortex tube containing **SMART Digest bulk resin**.

2

Aliquot 15 μ L of resin



Take 15 μ L of **SMART Digest bulk resin** (magnetic or non-magnetic) and add to an appropriate well (deep well plate, PCR plate, PCR strip, or 1.5 mL tube).

5

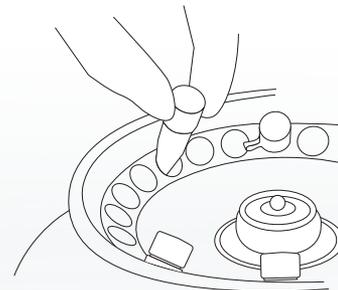
Digestion



Set your heater/shaker to **70 °C/1400 RPM**, allow to equilibrate for 5 minutes. Then add your samples for the required time for digestion.

6

Cleanup



Remove the SMART Digest tube from the heater/shaker and perform one of the sample cleanup methods based on the complexity of your starting matrix.

Note: For the magnetic bead option it is recommended that a SOLA μ SPE plate cleanup step is performed.



Note: Refer to the Digestion Optimization Section.



Note: Refer to the Post Digestion Process.

3

Add 150 μL of buffer



+ 150 μL

Add 150 μL of **SMART Digest buffer** to the well/tube containing the SMART Digest resin.

4

Add 50 μL of sample



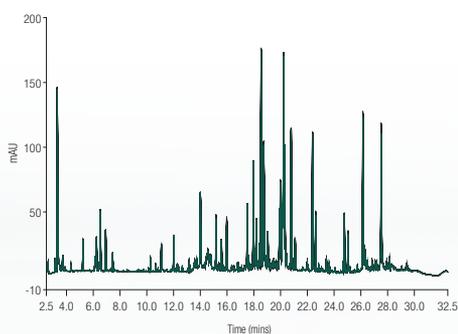
+ 50 μL

Take 50 μL of sample and add to the well/tube containing the **SMART Digest resin** (final volume of 200 μL per sample). Seal the well/tube.

Note: When using a deep well plate the use of cap mats are recommended (Thermo Scientific™ Matrix™ SeptraSeal caps P/N 4463). When using a PCR plate the use of iron-on seals are recommended.

7

Analyze



Analyze sample.

How to use the SMART Digest soluble kit

1

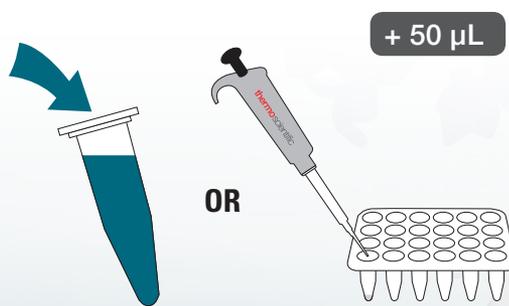
Add 150 μL of buffer



Add 150 μL of **SMART Digest buffer** to the well/tube.

2

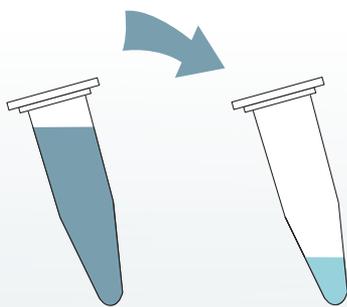
Add 50 μL of sample



Add 50 μL of **sample** to the well/tube (final volume of 200 μL per sample). Seal well/tube.

5

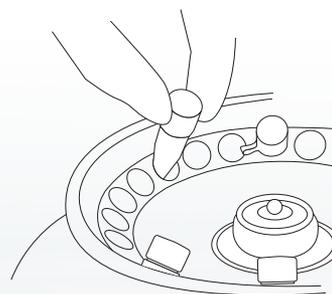
Quench



Remove sample tube/well from heater. Allow to cool. Remove 175 μL of sample and transfer to a tube containing 175 μL of 1% trifluoroacetic acid in water.

6

Cleanup



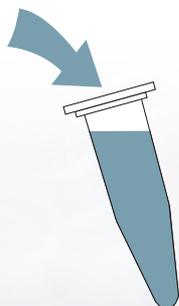
Perform one of the sample cleanup methods based on the complexity of your starting matrix.



Note: Refer to the Post Digestion Process.

3

Add 5 μ L of trypsin



+ 5 μ L

Add 5 μ L of **SMART Digest soluble trypsin**.

4

Digestion



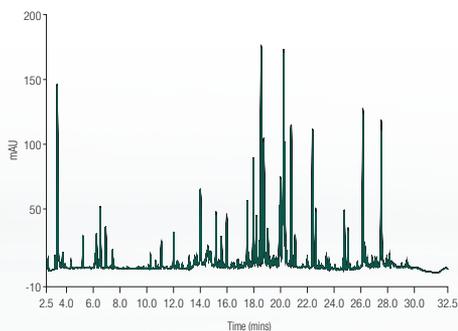
Set your heating apparatus to 70 °C (no shaking required) allow to equilibrate for 5 minutes. Then add your samples for the required time for digestion.



Note: Refer to the Digestion Optimization Section.

7

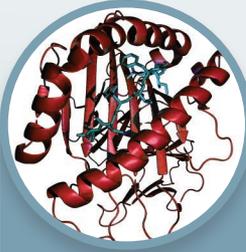
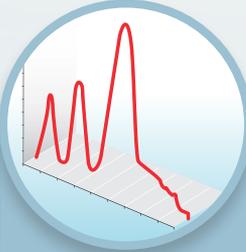
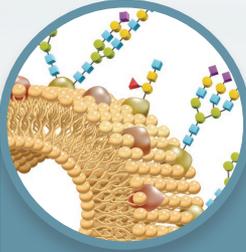
Analyze



Analyze sample.

Columns and Consumables for Biopharma

A full range of Thermo Scientific™ columns are available for Biopharma applications including affinity, ion exchange, reversed phase, hydrophobic interaction, and size exclusion.

	 Peptide Mapping	 Size Exclusion	 Charge Variant Analysis	 Intact Proteins	 Glycan Analysis
Detection	UV / UV-MS	UV / UV-MS	UV	UV / UV-MS	FLD and FLD-MS
Application goal	Confirm sequence	Check monomer versus aggregates	Check charge variation within antibody sample	Check purity of the antibody	Check glycan presence and structure
Recommended products	Acclaim PepMap column SMART Digest kit SOLA μ SPE EASY-Spray column	MABPac SEC-1 column	MABPac SCX-10 pH Gradient Buffer	MABPac RP column MABPac HIC column	GlycanPac AXH-1 and AXR-1 columns Accucore 150-Amide-HILIC column
Benefits	Retention reproducibility	Reproducible LC/MS compatible	Ultra-high resolution	Rugged Low carryover	High resolution More information

Upgrade your maps with a complete workflow for biotherapeutic protein characterization

Magnify the advantages that SMART Digest kits bring to your peptide mapping workflows, with time-saving UHPLC, MS and software technologies.

Faster and more reproducible separations

Engineered to build your drug pipeline

The Vanquish UHPLC family was built for biopharma characterization. High pressure bio-inert flow paths and SmartInject (intelligent sample pre-compression prior to injection) ensures high peak capacity, retention time stability and peak area precision - ideal for peptide mapping applications. Multiple thermostating options and adjustable gradient delay volume means that you can seamlessly transfer peptide mapping methods from any LC-MS to LC-UV as your drug candidate moves from development to routine QC.



Say goodbye to your Q-TOF

The Orbitrap you love now excels in both peptide mapping and intact mass analysis

Realize complete biopharmaceutical characterization with the new Thermo Scientific™ Q Exactive™ BioPharma platform. The platform offers new operational modes optimized for three protein characterization workflows; **High Mass Mode** for intact and native high molecular weight proteins and ADCs; **Protein Mode** for antibody fragments and spectral resolution; and **Standard Mode** that performs peptide mapping with unparalleled acquisition speed and accuracy. Go from vial to file with new mapping and intact protein software.



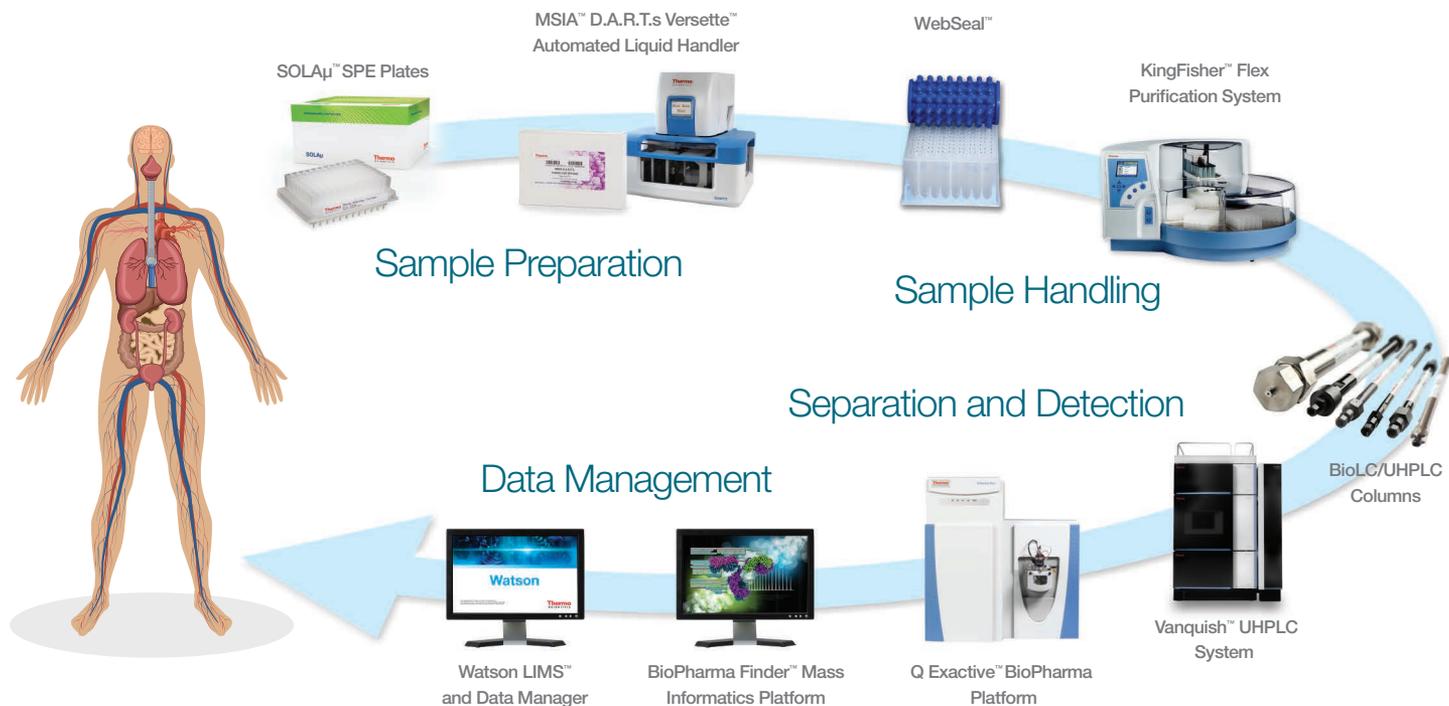
100% coverage peptide mapping and intact protein characterization in one package

The Thermo Scientific™ BioPharma Finder™ Software gives you the ability to perform sequence variant searching, disulfide link analysis, *de novo* sequencing with relative quantitation of post-translational modifications, and comparability studies. BioPharma Finder assures that you won't miss a thing in your sample. The multiple interactive plot display options mean you can see what you want, when you need it.



Characterize the full diversity of biomolecules

Thermo Scientific™ solutions for biomolecule separations



Find out more at thermofisher.com/SMARTdigest

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