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SMART Digest and SMART Digest ImmunoAffinity Kits

Smarter protein preparation

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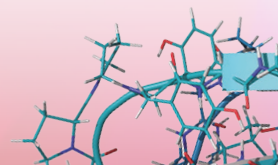
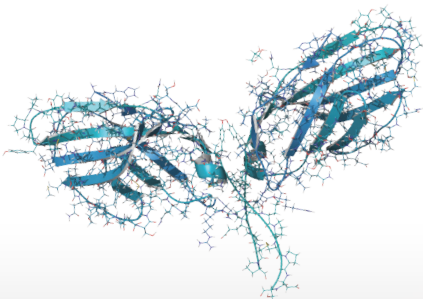
The Thermo Scientific™ SMART Digest™ and the SMART Digest ImmunoAffinity (IA) kits are designed for biomarker and bio-therapeutic characterization and quantitation. The kits deliver sample preparation of proteins that are:

- **Fast**
- **Simple**
- **Highly reproducible**
- **Sensitive**
- **Compatible with automation**

Challenges for biopharmaceutical laboratories

Modern biopharmaceutical laboratories are tasked with providing high quality quantitative and qualitative analytical results, often in high-throughput, regulated environments. This is exacerbated by the increase in efficacy of biotherapeutics and the often low levels of biomarkers in complex biological matrices.

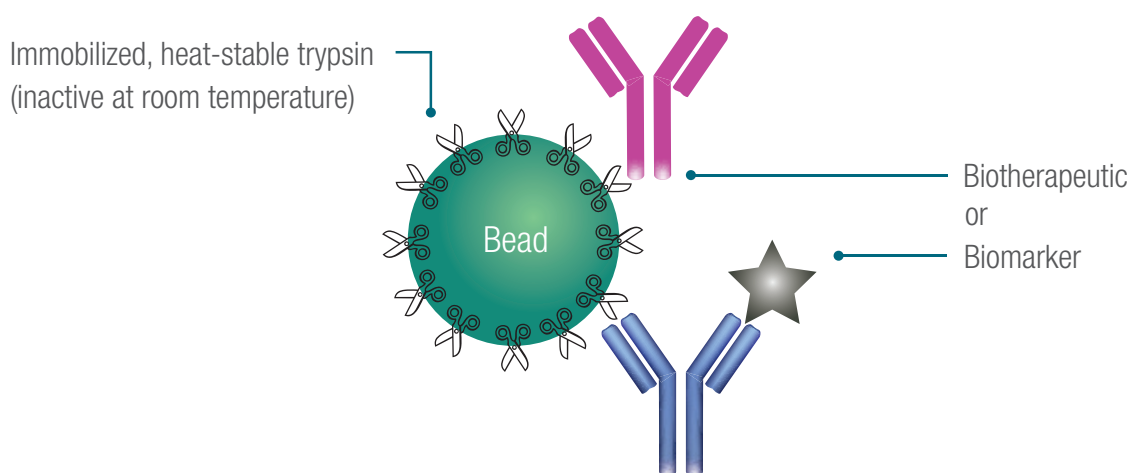
One of the key areas which affects these requirements is sample preparation. Current technologies employed are subject to high levels of irreproducibility, poor sensitivity, and protracted methodologies that are not amenable to automation and often require 24 hours to deliver results.



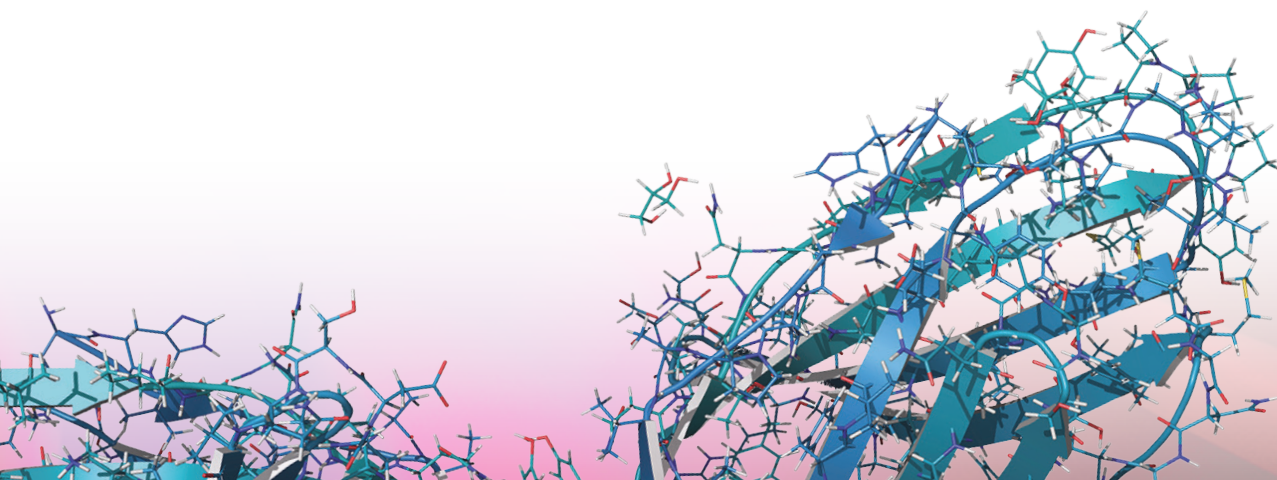
Addressing sample preparation challenges for biopharma

SMART Digest kits

The kits deliver fast, simple and highly reproducible digestion of proteins for characterization and quantitation applications, achieved due to the **heat-stable immobilized trypsin design**.

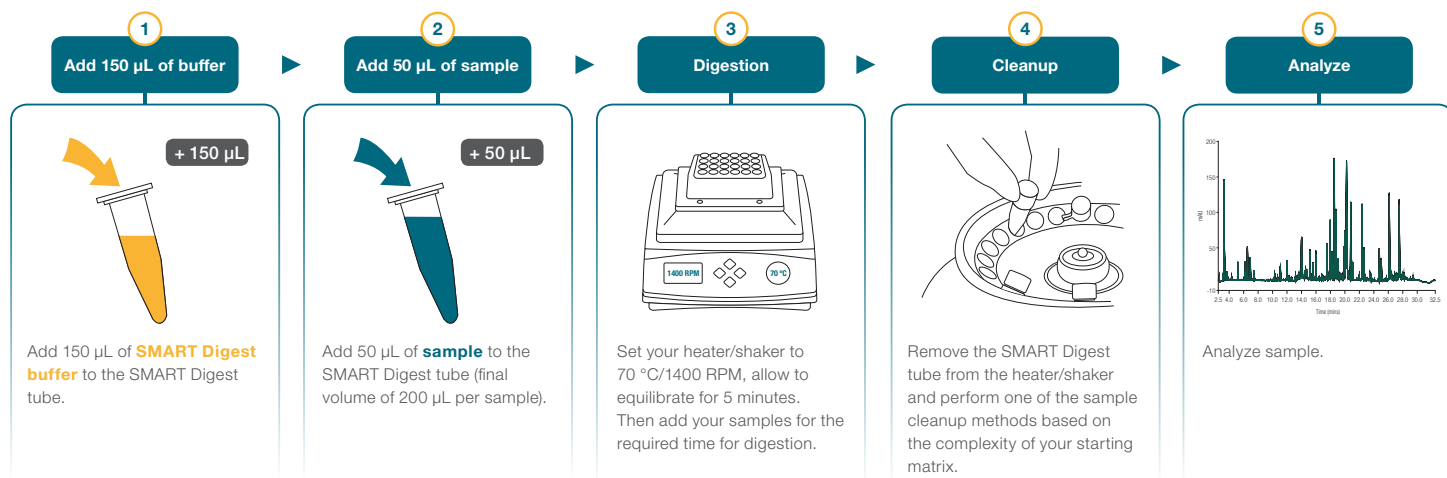


This design allows the digestion process to be significantly simplified compared to traditional in-solution digest protocols.



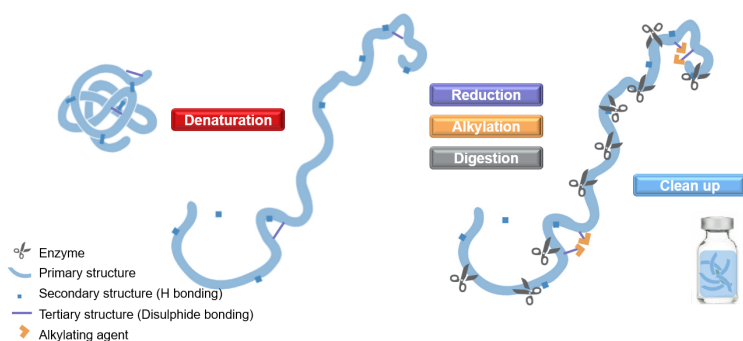
SMART Digest kit

Simple and easy-to-use workflow



Standard in-solution digest kit

Complex and laborious workflow



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).

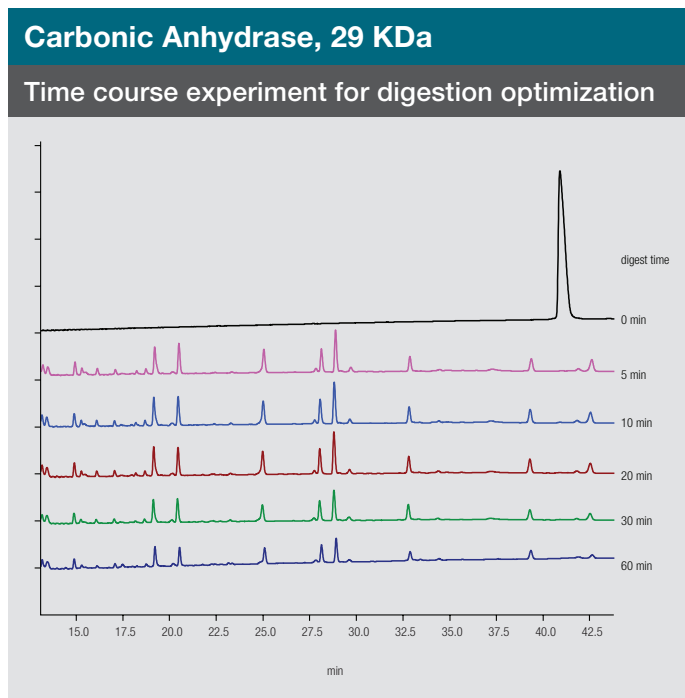
Q: Do I have to reduce and alkylate my protein?

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.

SMART Digest kits

Fast digestion of proteins

Using the SMART Digest kit simplifies the process and reduces the time needed for sample preparation. This not only speeds up your workflows, but also significantly reduces method development time. In the example below it can be seen that for carbonic anhydrase full digestion is achieved in 5 minutes.



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

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 (PTMs)?

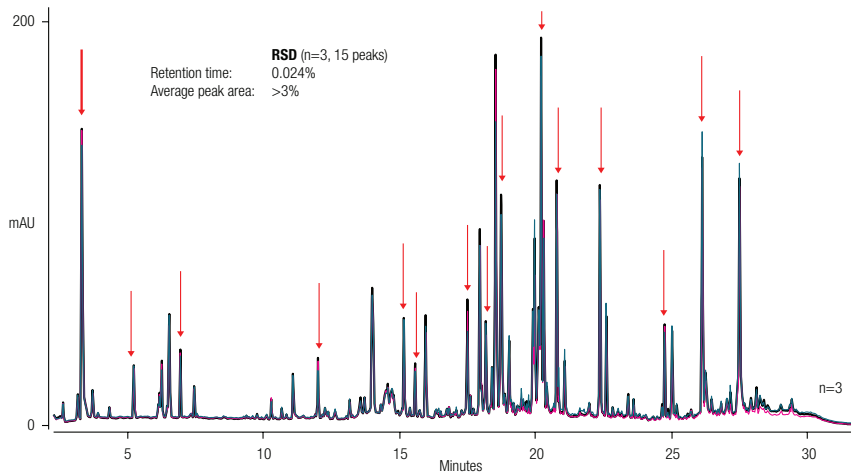
In comparison to in-solution digests a comparable number of PTMs have been observed when screening for deamidation, amidation, methylation and oxidation. No modifications to existing PTMs, such as phosphorylated sites, have been observed.

SMART Digest kits

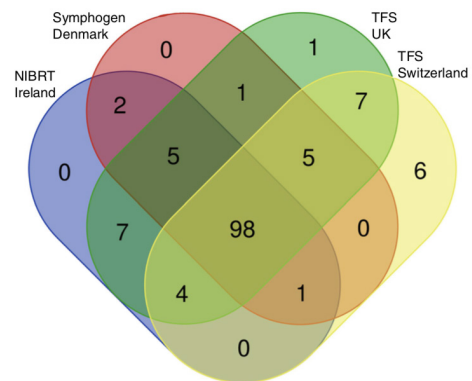
High quality analytical results

The SMART Digest kit provides significant improvements in reproducibility over existing protocols, which results in fewer sample failures, higher throughput and the ability to more easily interrogate data. This allows for reproducible results user-to-user, day-to-day and lab-to-lab. The

ultra-violet (UV) chromatogram on the below shows overlays from three separate SMART digestions from the same monoclonal antibody (mAb), conducted by three individual operators, with retention time RSD of 0.024%



The following study highlights the reproducibility of results when transferring a method between 4 laboratories in different locations (UK, Ireland, Denmark and Switzerland). NIST mAb samples digested in each laboratory produced virtually identical peptide Maps. 98 peptides were found in all laboratories with only a few additional peptides found in individual laboratories at low levels of abundance.



Venn diagram of the peptides identified from automated NISTmAb trypsin digestions performed in four different laboratories. Peptide lists include all the peptides within ± 5 ppm accuracy and including up to one missed cleavage peptides

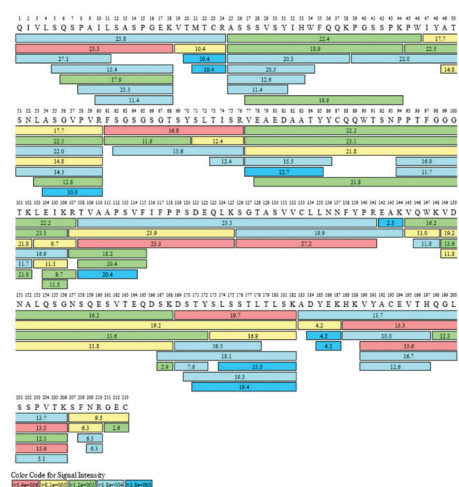
Characterization

The SMART Digest kit, in combination with the high resolution accurate mass (HRAM) capabilities of the Thermo Scientific™ Q Exactive™ mass spectrometers, and high resolution chromatographic separation with the Vanquish UHPLC systems, provides high quality data sets that can be more effectively interrogated with Thermo Scientific™ BioPharma Finder™ Software.

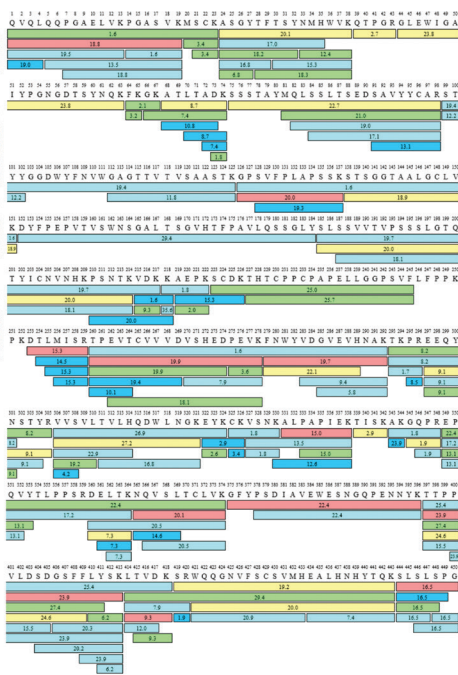
The BioPharma Finder software allows us to map our protein, in the case for Rituximab ~100% sequence coverage is achieved, as well as quantitation of modifications on both the light and heavy chains.

This is of particular importance in understanding how the molecule will interact within a biological system.

Rituximab light chain

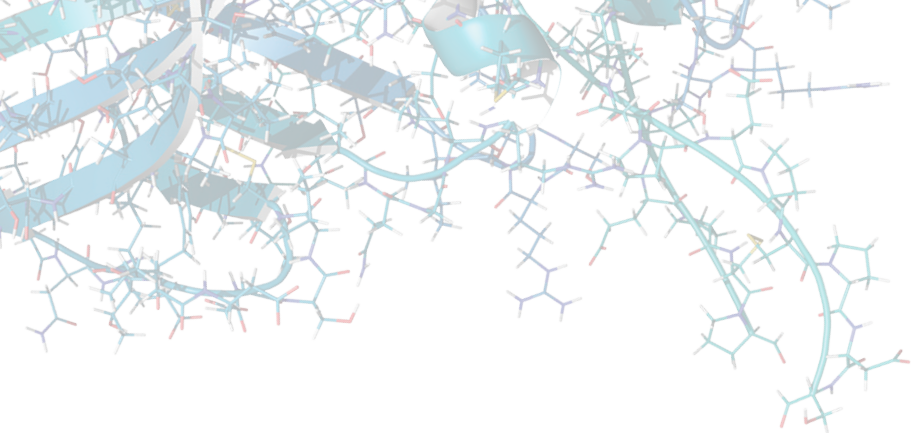


Rituximab heavy chain



Sequence coverage ~100%

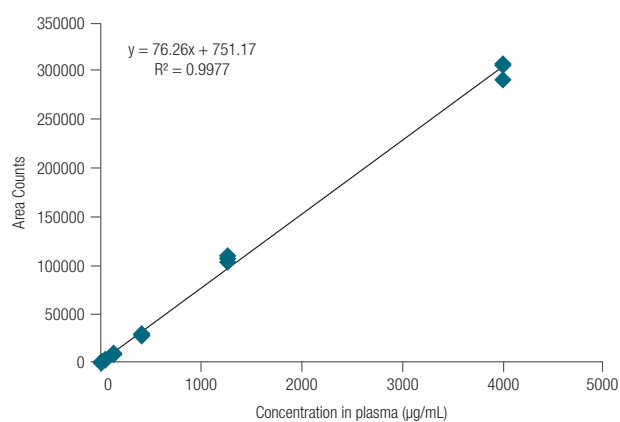
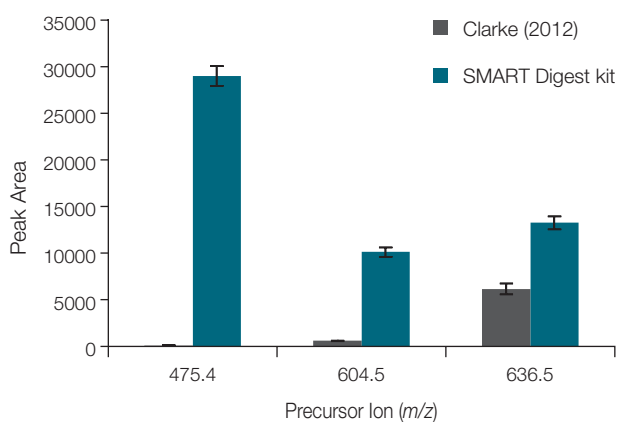
Protein	Modification	Recovery	Abundance
Rituximab_LC	Q1+NH ₃ loss	Good	87.81%
Rituximab_LC	W90+Oxidation	Good	2.06%
Rituximab_HC	~Q1+NH ₃ loss	Good	100.00%
Rituximab_HC	W281+Oxidation	Good	4.98%
Rituximab_HC	N301+A1G0F	Fair	2.87%
Rituximab_HC	N301+A1G1F	Fair	1.22%
Rituximab_HC	N301+A2G0	Fair	1.30%
Rituximab_HC	N301+A2G0F	Fair	37.69%
Rituximab_HC	N301+A2G1F	Fair	44.86%
Rituximab_HC	N301+A2G2F	Fair	10.77%
Rituximab_HC	N301+M5	Fair	1.07%
Rituximab_HC	N365+Deamidation	Good	2.72%
Rituximab_HC	W385+Oxidation	Good	5.37%
Rituximab_HC	G450+Lys	Good	3.2683%



Quantitation

The SMART Digest kit allows confident detection of biomarkers with high sensitivity within a wide dynamic range, as can be seen below with the example of

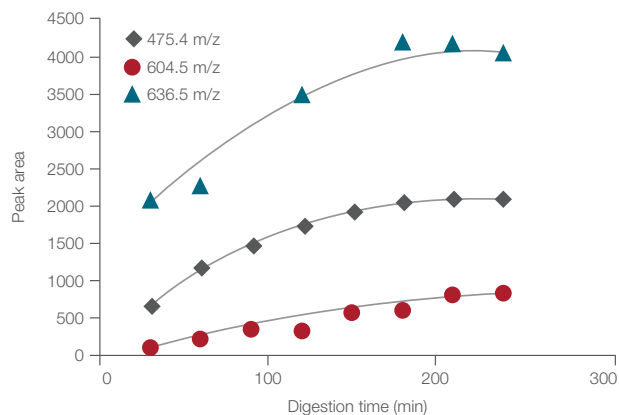
thyroglobulin. Greater sensitivity is achieved in 3.5 hours compared to an in-solution digest protocol taking 20 hours to complete.



Measurement of serum thyroglobulin after tryptic digestion of serum samples.

- SMART Digest kit: 25% plasma, 3.5 h digestion
 - In-solution digest: 20% plasma, R/A, 4 + 16 h digestion
- Clarke *et al.* (2012), J. Investigative Medicine, 60(8)

Calibration curve for thyroglobulin signature peptide in murine plasma (4–4000 µg/mL).



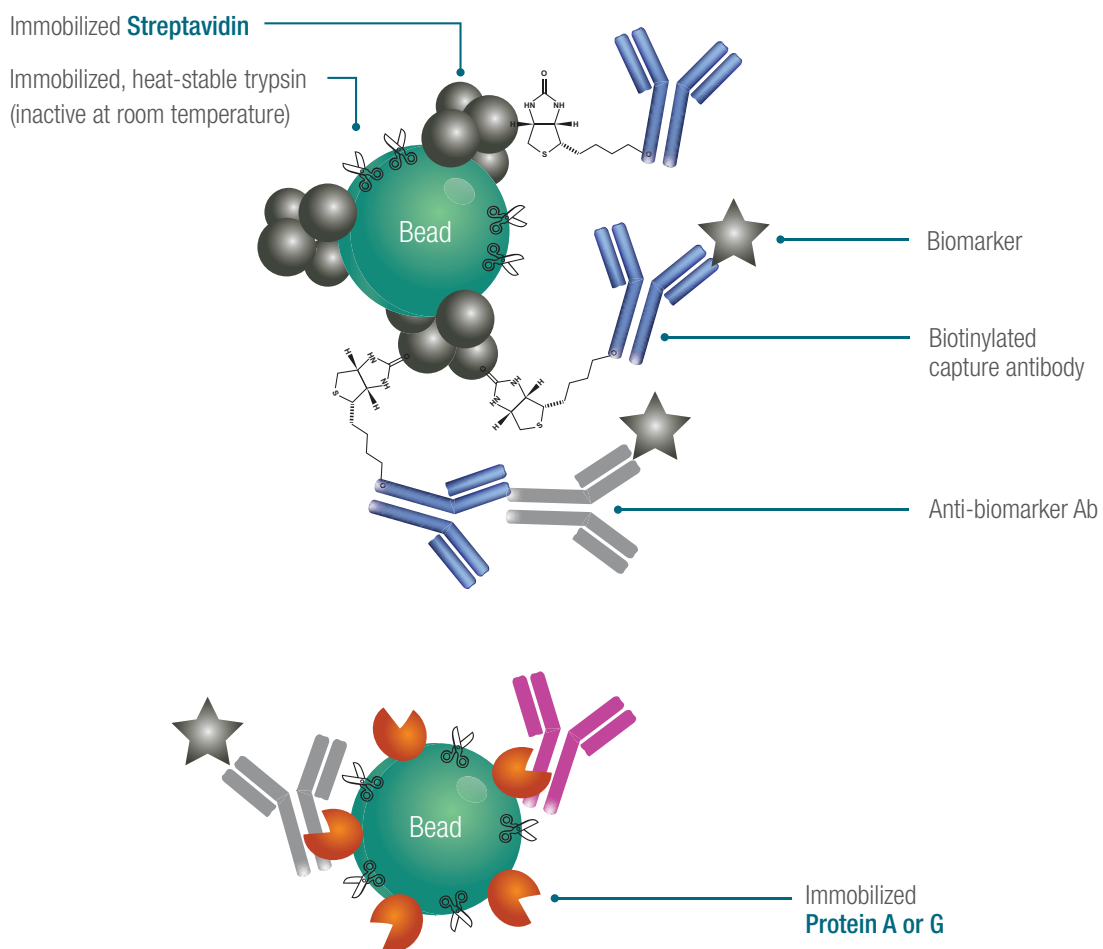
Digestion time curve 70 °C. Optimum digestion time of 3.5 hours for thyroglobulin was determined by monitoring the peak area response for the three signature peptides.

SMART Digest ImmunoAffinity kits

SMART Digest IA kits have all the advantages previously outlined for fast, easy and reproducible protein digestion for quantitation and characterization applications with the added advantage of combining an immunocapture and the digestion process into a single well.

This has significant benefits for quantitation studies where immunoaffinity capture is typically employed to increase sensitivity by purifying low level proteins from complex biological matrices. This step is then followed by protein digestion.

The SMART Digest IA kits achieve this due to their unique design where the immunoaffinity reagents (either streptavidin, protein A or protein G) and heat activated thermally stable trypsin are co-immobilized onto a single bead. Following the binding of a capture reagent to the bead, and enrichment of the target, the enzyme is activated at elevated temperatures for accelerated digestion under protein denaturing conditions. The resulting workflow is as easy as enrich, wash and digest. Magnetic and non-magnetic versions of the beads are available.

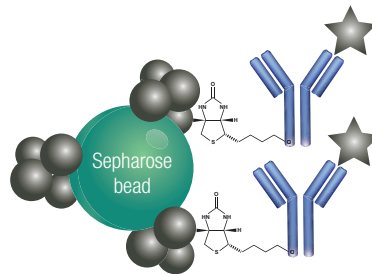


How does the SMART Digest IA kit work?

Conventional protein enrichment and digestion

Expose biotinylated capture antibody to biological sample

Incubate with affinity capture resin



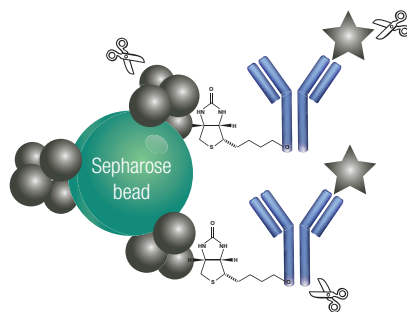
Conventional tryptic digest

Reduction, TCEP: 60 °C, 30–60 min

Alkylation, IAA: RT, 30 min

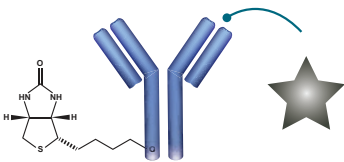
Quenching, ACC: RT, 15 min

Digestion: 37 °C, 12–16 h

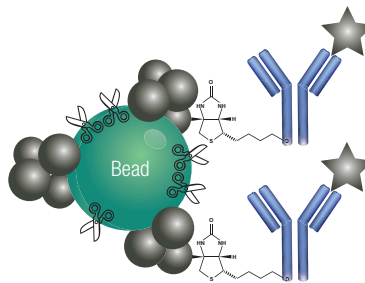


Total time
15–24 hours

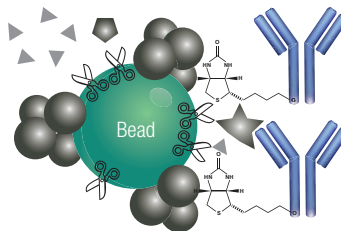
Protein enrichment and digestion using the SMART Digest IA kit



Use SMART Digest IA resin for affinity capture.
Incubate at room temperature.



Digestion with SMART Digest IA resin
70 °C, 1 h



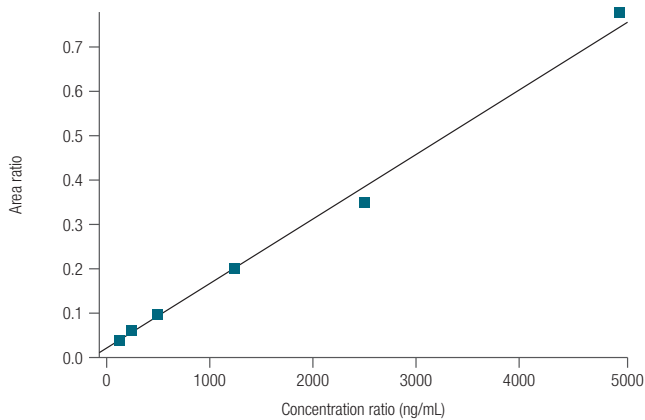
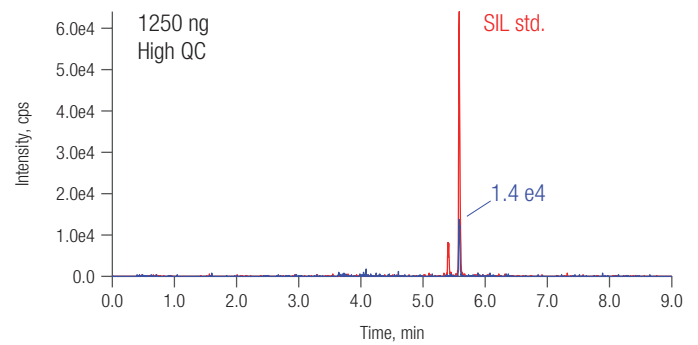
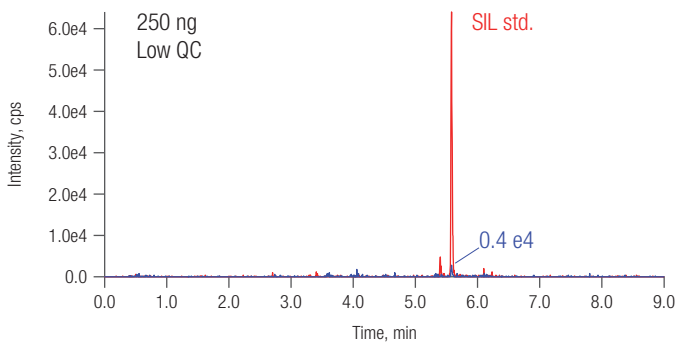
Total time
3–4 hours

Quantitation with the SMART Digest IA kit compared to a conventional approach

The following is an example of using the SMART Digest IA Streptavidin kit for the quantitation of a biomarker (human interferon α 14) in human plasma, compared to a traditional immunocapture and digestion method.

The SMART Digest IA protocol used involved an immunocapture step, taking 2 hours, followed by a 1 hour, high-temperature digestion with immobilized trypsin. This is compared to immunocapture with a high capacity streptavidin gel followed by overnight tryptic digestion of the biomarker protein. A SIL peptide was spiked into the samples to act as an internal standard.

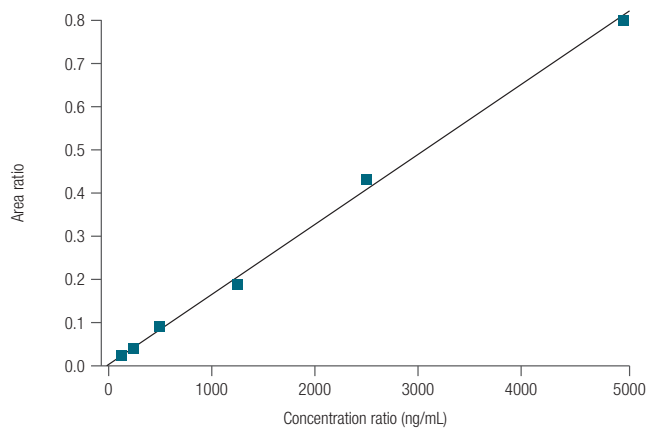
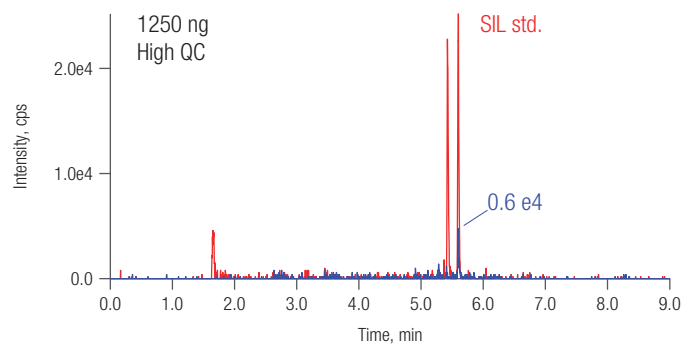
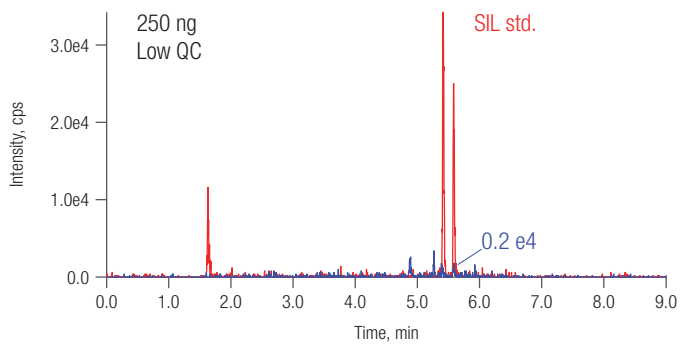
SMART Digest IA kit process



Standard curve (n = 1)			Quality controls (n = 4)		
Actual conc (ng/mL)	Accuracy (%)	Calc value (ng/mL)	Actual conc (ng/mL)	CV (%)	Accuracy (%)
125	93	116.5			
250	107	266.3	250	11.5	90.2
500	106	531.1			
1250	100	1247	1250	7.4	99.1
2500	90	2251			
5000	104	2251			

Recovery with SMART Digest IA kit	
500 ng/mL spike	7330 (cps)
Recovery	64%

Conventional streptavidin agarose process



Standard curve (n = 1)			Quality controls (n = 4)		
Actual conc (ng/mL)	Accuracy (%)	Calc value (ng/mL)	Actual conc (ng/mL)	CV (%)	Accuracy (%)
125	105	131			
250	90	225	250	14.5	111.2
500	109	544			
1250	92	1149	1250	4.1	104.8
2500	106	2654			
5000	99	4922			

Recovery with conventional approach	
500 ng/mL spike	2778 (cps)
Recovery	35%

Note: Data provided courtesy of PharmaCadence Analytical Services, Hatfield, PA, USA. Carmen Fernández-Metzler, Bonnie Baker, Robyn Buerger.

A step change in protein affinity capture and digestion

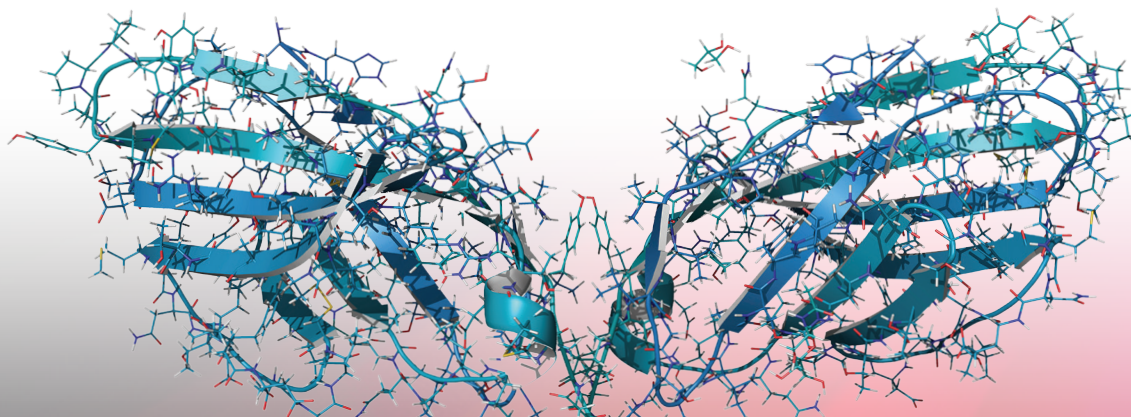
SMART Digest and SMART Digest IA kits provide a significant change in protein sample preparation, by delivering workflows that are:

- Significantly faster
- Easier to use
- Highly reproducible
- Sensitive
- Compatible with automation

Ordering information

SMART Digest kits for peptide mapping

Description	Part number
SMART Digest Trypsin Kit with collection plate	60109-101
SMART Digest Trypsin Kit, bulk resin option	60109-101-B
SMART Digest Trypsin Kit, magnetic bulk resin option	60109-101-MB
SMART Digest Trypsin Kit with filter/collection plate	60109-102
SMART Digest Trypsin Kit, bulk resin option with filter/collection plate	60109-102-B
SMART Digest Trypsin Kit, magnetic bulk resin option with filter/collection plate	60109-102-MB
SMART Digest Trypsin Kit with SOLA μ /collection plate	60109-103
SMART Digest Trypsin Kit, bulk resin option with SOLA μ /collection plate	60109-103-B
SMART Digest Trypsin Kit, magnetic bulk resin option with SOLA μ /collection plate	60109-103-MB
SMART Digest Soluble Trypsin Kit	60113-101
SMART Digest Chymotrypsin Kit with collection plate	60109-104
SMART Digest Chymotrypsin Kit, bulk resin option	60109-104-B
SMART Digest Chymotrypsin Kit, magnetic bulk resin option	60109-104-MB
SMART Digest Chymotrypsin Kit with filter/collection plate	60109-105
SMART Digest Chymotrypsin Kit, bulk resin option with filter/collection plate	60109-105-B
SMART Digest Chymotrypsin Kit, magnetic bulk resin option with filter/collection plate	60109-105-MB
SMART Digest Chymotrypsin Kit with SOLA μ /collection plate	60109-106
SMART Digest Chymotrypsin Kit, bulk resin option with SOLA μ /collection plate	60109-106-B
SMART Digest Chymotrypsin Kit, magnetic bulk resin option with SOLA μ /collection plate	60109-106-MB
SMART Digest Proteinase K Kit with collection plate	60109-107
SMART Digest Proteinase K Kit, bulk resin option	60109-107-B
SMART Digest Proteinase K Kit, magnetic bulk resin option	60109-107-MB
SMART Digest Proteinase K Kit with filter/collection plate	60109-108
SMART Digest Proteinase K Kit, bulk resin option with filter/collection plate	60109-108-B
SMART Digest Proteinase K Kit, magnetic bulk resin option with filter/collection plate	60109-108-MB
SMART Digest Proteinase K Kit with SOLA μ /collection plate	60109-109
SMART Digest Proteinase K Kit, bulk resin option with SOLA μ /collection plate	60109-109-B
SMART Digest Proteinase K Kit, magnetic bulk resin option with SOLA μ /collection plate	60109-109-MB
SMART Digest Pepsin Kit with collection plate	60109-110
SMART Digest Pepsin Kit, bulk resin (no collection plate)	60109-110-B
SMART Digest Pepsin Kit with magnetic bead, bulk option (no collection plate)	60109-110-MB
SMART Digest Pepsin Kit with filter and collection plate	60109-111
SMART Digest Pepsin Kit with SOLA μ collection plate	60109-112



SMART Digest low pH kits for MAM

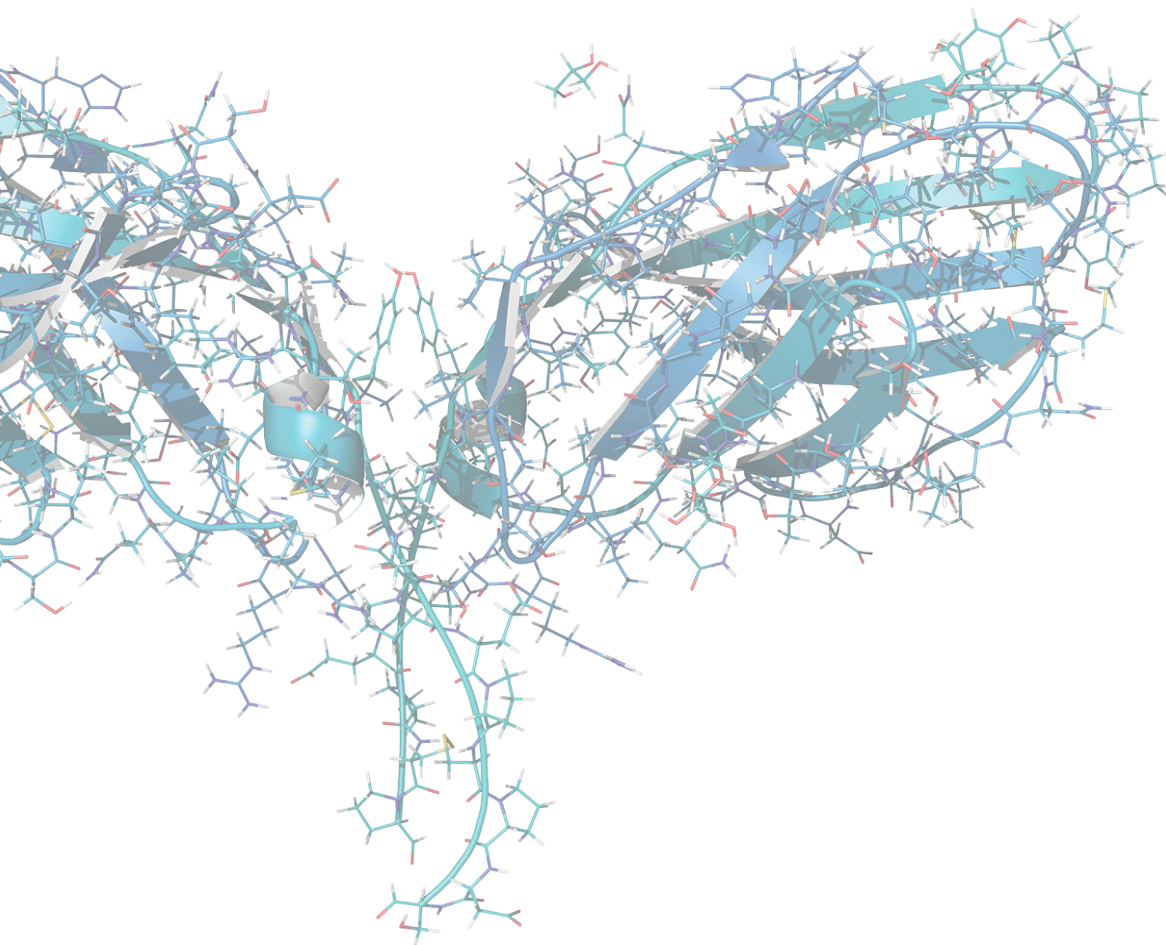
Description	Part number
SMART Digest low pH Kit Trypsin	60109-101-LPH
SMART Digest low pH Kit Trypsin magnetic beads	60109-101-MB-LPH
SMART Digest KIT Chymotrypsin magnetic bulk low pH buffer	60109-104-MB-LPH
SMART Digest low pH Kit Trypsin magnetic beads	60109-101-MB-LPH
SMART Digest Kit Chymotrypsin magnetic bulk low pH buffer	60109-104-MB-LPH

SMART Digest IA and bulk fractionation kits for peptide quantitation

Description	Part number
SMART Digest IA Kit, Streptavidin non-magnetic	60110-101
SMART Digest IA Kit, Streptavidin non-magnetic with SOLA μ SPE and collection plate	60110-102
SMART Digest IA Kit, Streptavidin magnetic with SOLA μ SPE and collection plate	60110-103
SMART Digest IA Kit, Streptavidin magnetic	60110-104
SMART Digest IA Kit, Protein A non-magnetic	60111-101
SMART Digest IA Kit, Protein A non-magnetic with SOLA μ SPE and collection plate	60111-102
SMART Digest IA Kit, Protein A magnetic with SOLA μ SPE and collection plate	60111-103
SMART Digest IA Kit, Protein A magnetic	60111-104
SMART Digest IA Kit, Protein G non-magnetic	60112-101
SMART Digest IA Kit, Protein G non-magnetic with SOLA μ SPE and collection plate	60112-102
SMART Digest IA Kit, Protein G magnetic with SOLA μ SPE and collection plate	60112-103
SMART Digest IA Kit, Protein G magnetic	60112-104
SMART Digest Bulk Protein A Fractionation Kit, non-magnetic, soluble Trypsin	60114-101
SMART Digest Bulk Protein A Fractionation Kit, non-magnetic, with SOLA μ collection plate, soluble Trypsin	60114-102
SMART Digest Bulk Protein A Fractionation Kit, magnetic, with SOLA μ collection plate, soluble Trypsin	60114-103
SMART Digest Bulk Protein A Fractionation Kit, magnetic, soluble Trypsin	60114-104
SMART Digest Bulk Protein A Fractionation Kit, magnetic, standalone	60116-101
SMART Digest Bulk Fractionation Kit, Protein G, non-magnetic, soluble Trypsin	60115-101
SMART Digest Bulk Fractionation Kit, Protein G, non-magnetic, with SOLA μ collection plate, soluble Trypsin	60115-102
SMART Digest Bulk Fractionation Kit, Protein G, magnetic, with SOLA μ collection plate, soluble Trypsin	60115-103
SMART Digest Bulk Fractionation Kit, Protein G, magnetic, soluble Trypsin	60115-104
SMART Digest Bulk Fractionation Kit, Protein G, magnetic, standalone	60117-101
SMART Digest Bulk Fractionation Kit, Streptavidin, magnetic, without soluble Trypsin	60118-101
SMART Digest Bulk Fractionation Kit, Streptavidin, magnetic, SOLA μ collection plate, with soluble Trypsin	60119-103
SMART Digest Bulk Fractionation Kit, Streptavidin, magnetic, with soluble Trypsin	60119-104

Complimentary products

Description	Part number
Thermo Scientific™ HyperSep™ Vacuum Manifold for HyperSep 96-Well Plates	60103-351
Thermo Scientific™ HyperSep™ Glass Block Vacuum Manifold Pump, North American version	60104-243
Thermo Scientific™ HyperSep™ Glass Block Vacuum Manifold Pump, European version	60104-241
Thermo Scientific™ SOLA μ ™ HRP SPE Plate	60209-001
KingFisher Duo Prime Purification System	5400110
Thermo Scientific™ KingFisher™ Flex Purification System	5400630



Expect reproducible results with sample prep, columns and vials



Find out more at thermofisher.com/smartdigest

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