

# Protein digestion for peptide mapping and quantitation

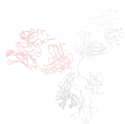
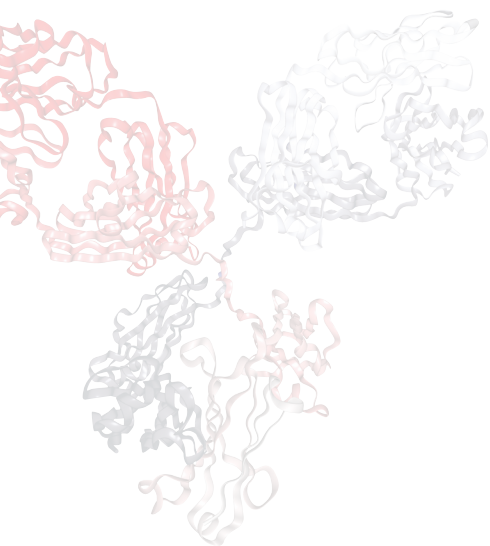
Overview of SMART Digest and  
SMART Digest ImmunoAffinity Kits



# SMART Digest and SMART Digest ImmunoAffinity kits

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

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



# SMART Digest kits

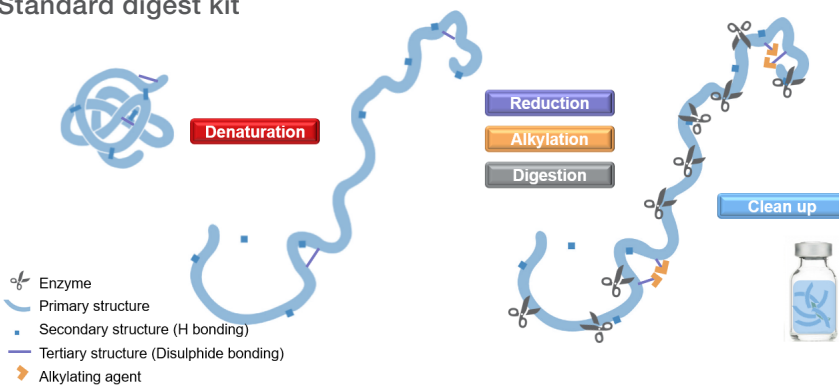
SMART Digest kits improve workflows by quickly and efficiently digesting proteins for characterization and quantitation applications, achieved due to the heat-stable immobilized trypsin design.

## Simplicity of use

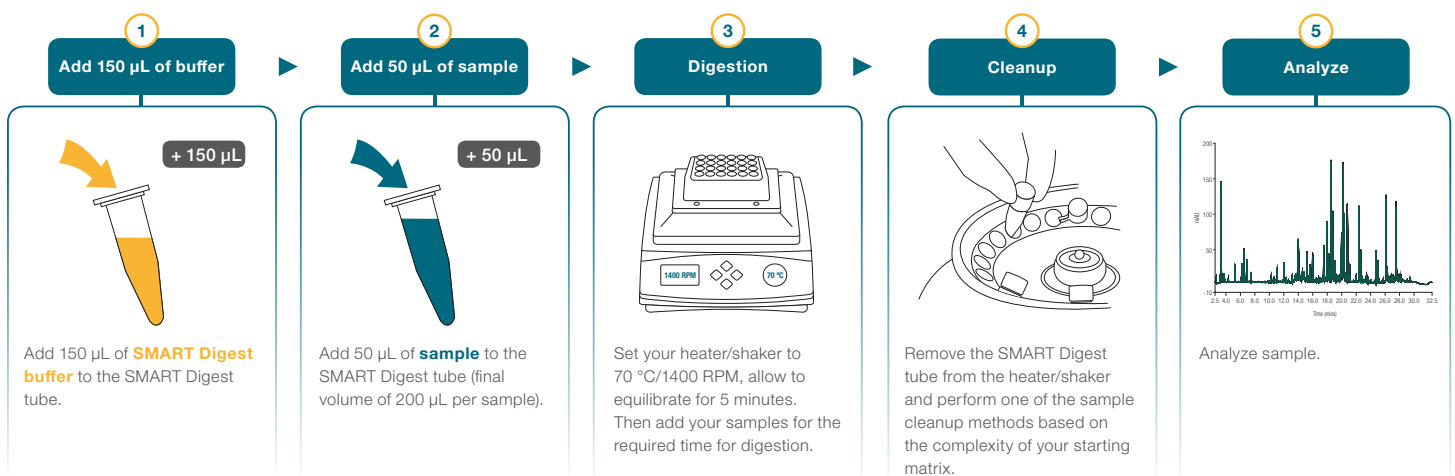
Current sample preparation protocols for the digestion of proteins are multifaceted and laborious. Without a single source solution, errors are far more likely to occur. Therefore, the overall protocol is subject to irreproducibility and significant sample processing time.

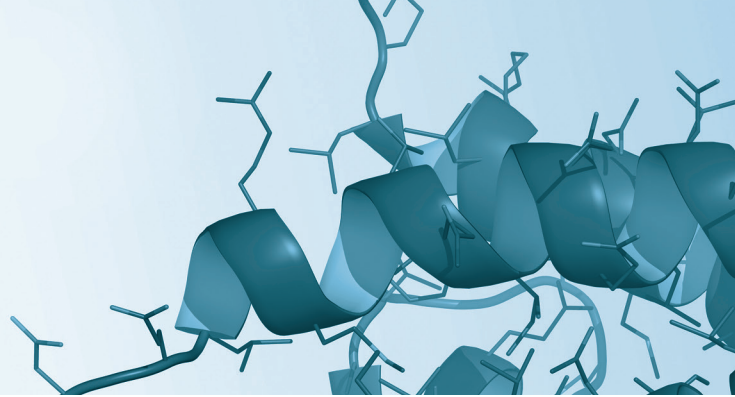
The SMART Digest kit overcomes these barriers by providing a simple to implement, integrated process (see the diagram below), which provides high data confidence and significantly increased reproducibility. The entire protocol can be easily automated for high throughput processing.

## Standard digest kit



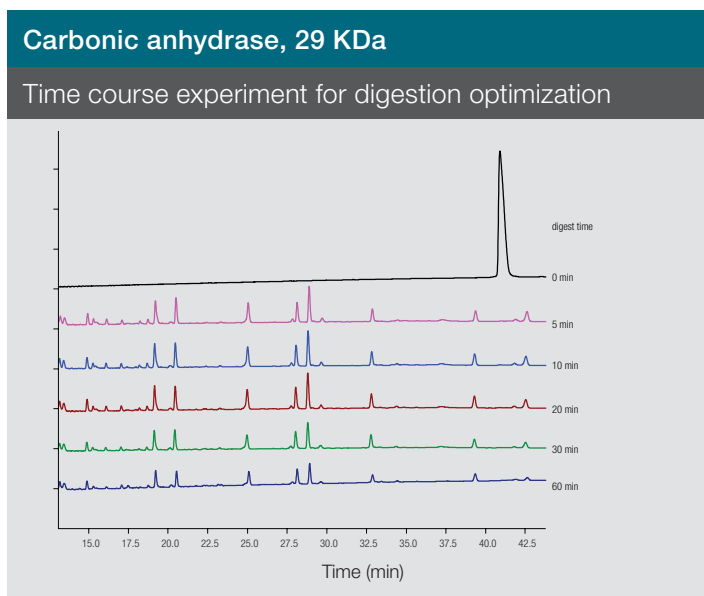
## SMART Digest kit





## Fast digestion

The SMART Digest process is not only simple, but significantly reduces the time from sample preparation to analysis. Typically it takes less than 60 minutes, dependent on sample complexity, to achieve full digestion. In the example below, we can see that carbonic anhydrase undergoes complete digestion in less than 5 minutes, as the intact protein peak at around 5 minutes is no longer present, whilst in later chromatograms, no further peaks appear following longer digestion times. The optimized digestion times of some common proteins are shown in the table adjacent. For proteins held together with disulfide bonds, 5 mM TCEP can be added to aid digestion.

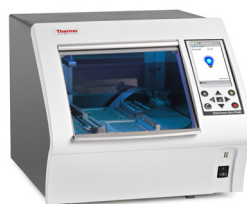


Typical digestion times	
Protein	Digest time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	30
IgG in 50 $\mu$ L plasma*	75
Ribonuclease A	30

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

## Automation

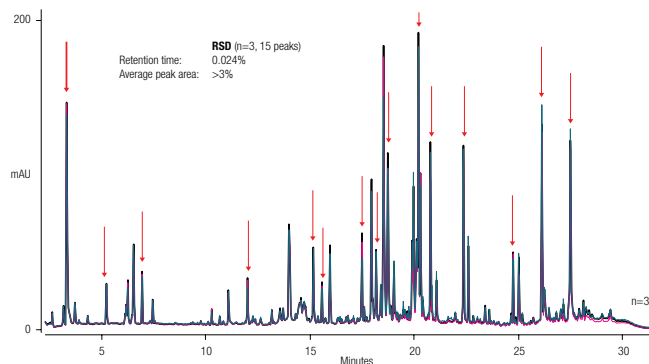
Due to their simple workflow, SMART Digest kits, SMART Digest IA kits and Magnetic SMART Digest kits are easy to automate with platforms such as the Thermo Scientific™ KingFisher™ Duo Prime purification System.



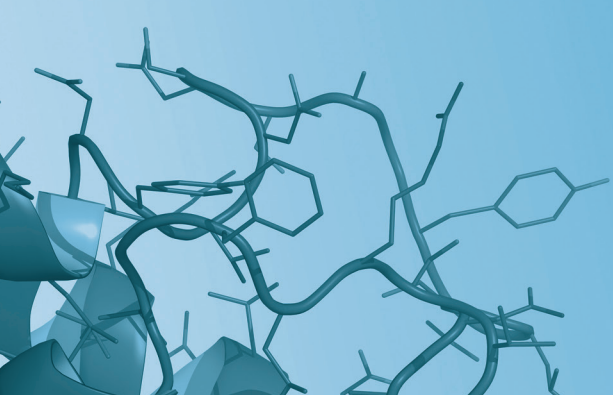
KingFisher Duo Prime purification system

## Reproducibility of digestion

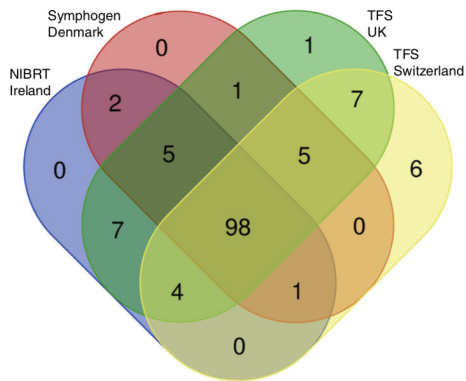
The SMART Digest kit provides significant improvements in reproducibility over existing protocols, which results in fewer sample failures, higher throughput and the ability to more easily interrogate data. This allows for reproducible results user-to-user, day-to-day and lab-to-lab. The ultra-violet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



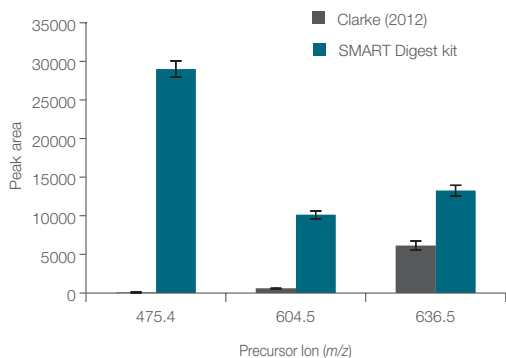
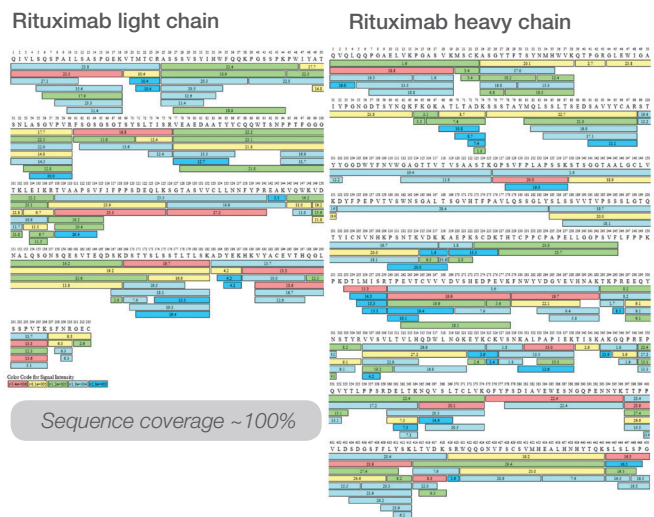
Venn diagram of the peptides identified from automated NISTmAb trypsin digestions performed in four different laboratories. Peptide lists include all the peptides within  $\pm 5$  ppm accuracy and including up to one missed cleavage peptides

### Mass spectrometry

Thermo Scientific™ Orbitrap Exploris™ 240 and 480 Mass Spectrometers add superior denatured and native mass spectrometry (MS) intact analysis and subunit top/middle-down analysis capabilities to one of the most powerful benchtop peptide mapping instruments available. When combined with Thermo Scientific™ BioPharma Finder™ Software it provides a complete integrated hardware and software solution for biopharmaceutical characterization.

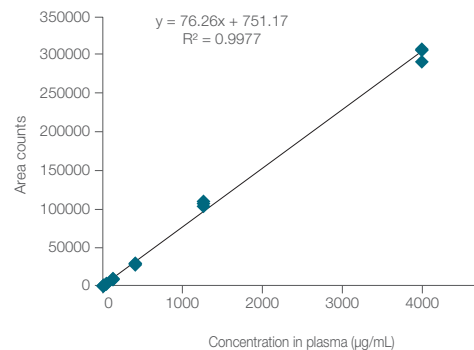
### Quantitation

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.

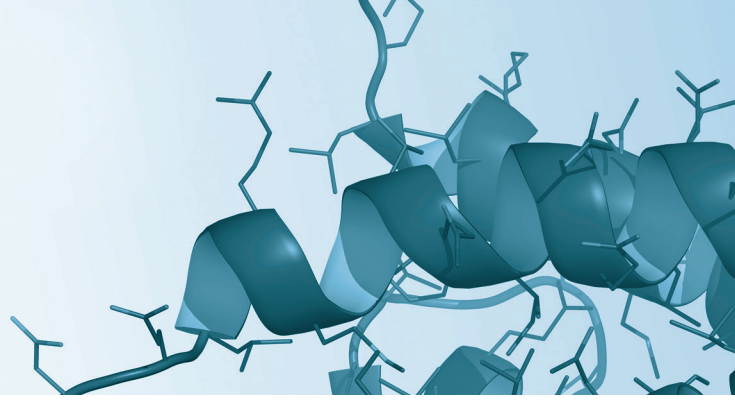


Measurement of serum thyroglobulin after tryptic digestion of serum samples.

- 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  $\mu\text{g/mL}$ ).



# SMART Digest ImmunoAffinity kits

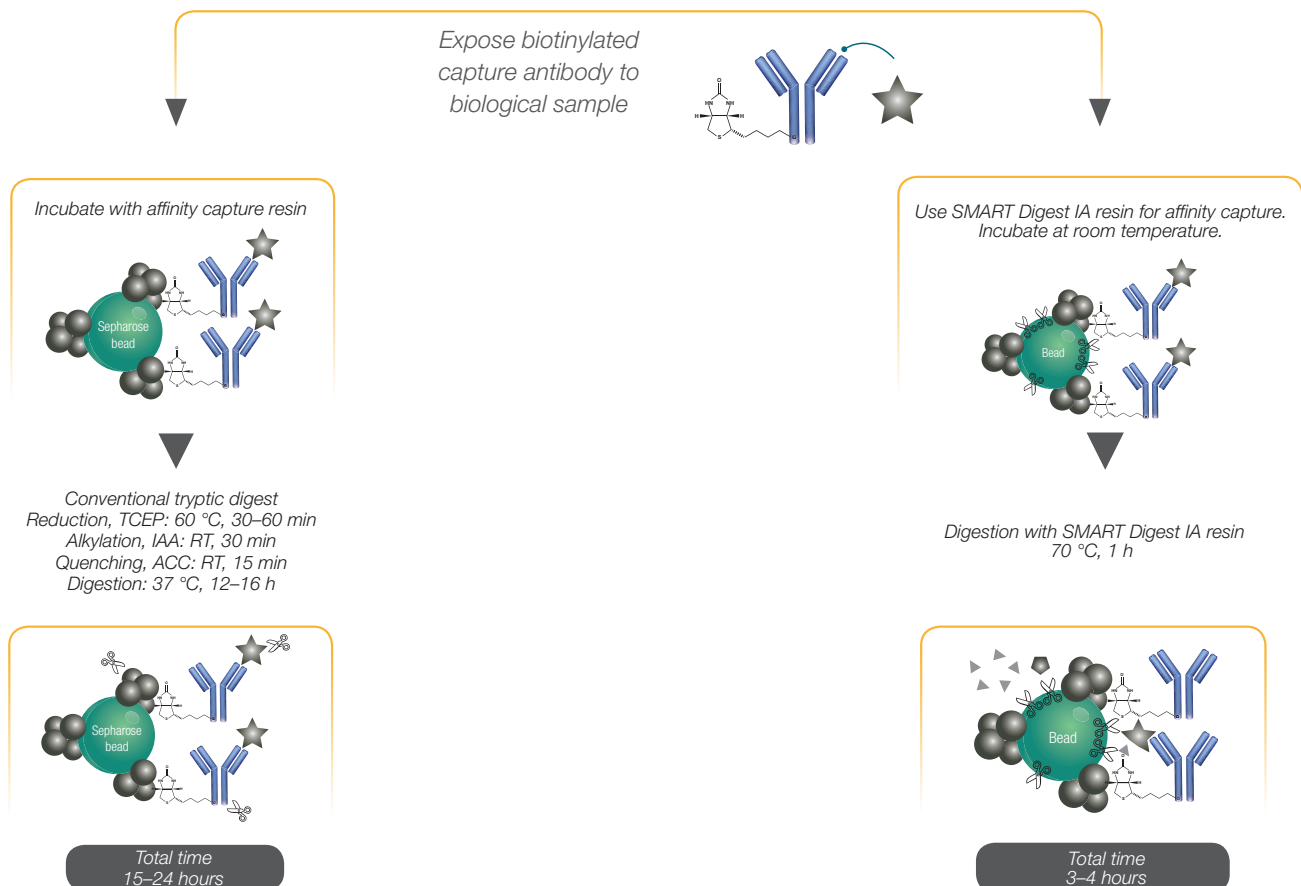
SMART Digest IA kits have all the advantages previously outlined for fast, easy and reproducible protein digestion for quantitation and characterization applications with the added advantage of combining an immunocapture and digestion process into a single well. This has significant benefits for quantitation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

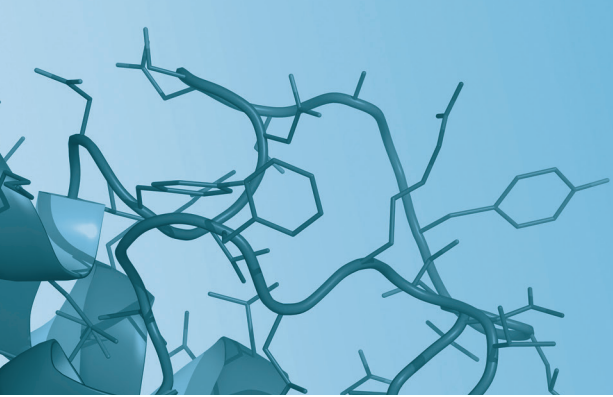
SMART Digest IA kits achieve this due to their unique design where the immunoaffinity reagents (either streptavidin, protein A or protein G) and heat activated thermally stable trypsin are co-immobilized onto a single bead. Following the binding of a capture reagent to the bead, and enrichment of the 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*

*Protein enrichment and digestion using the SMART Digest IA kit*



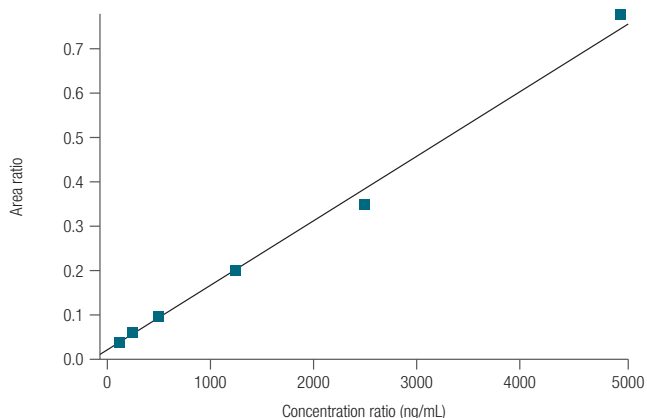
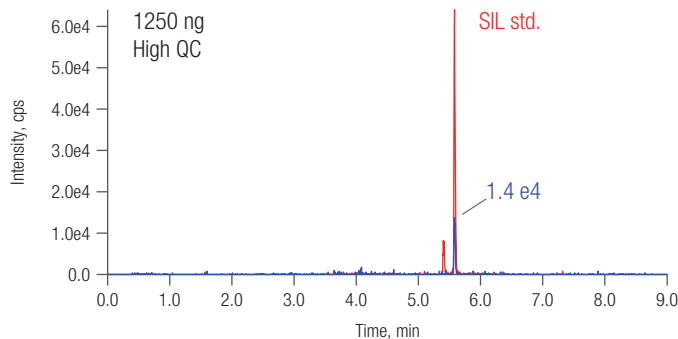
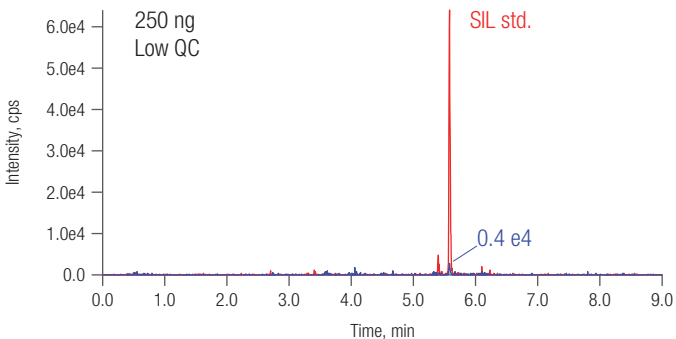


### Quantitation with the SMART Digest IA kit compared to a conventional approach

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

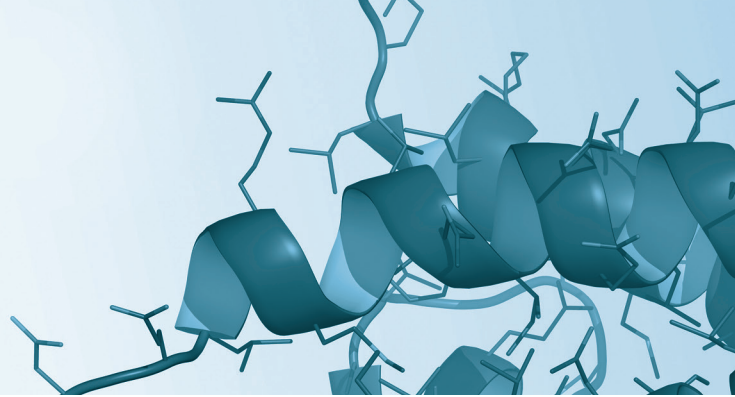
The SMART Digest IA protocol used involved an immunocapture step, which took 2 hours, followed by a 1 hour, high-temperature digestion with immobilized trypsin. This is compared to immunocapture with a high capacity streptavidin gel followed by overnight tryptic digestion of the biomarker protein. A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

### SMART Digest IA kit process

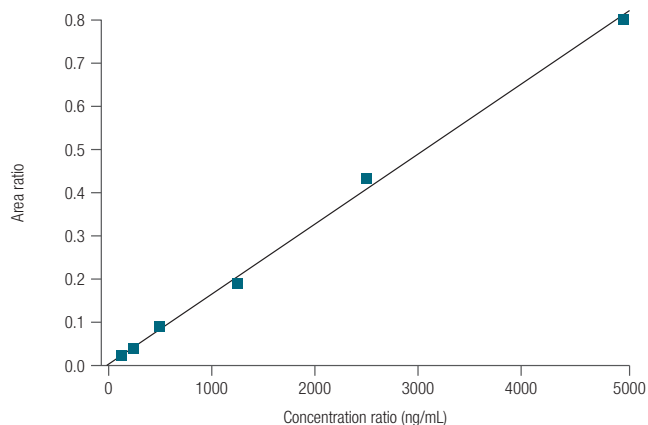
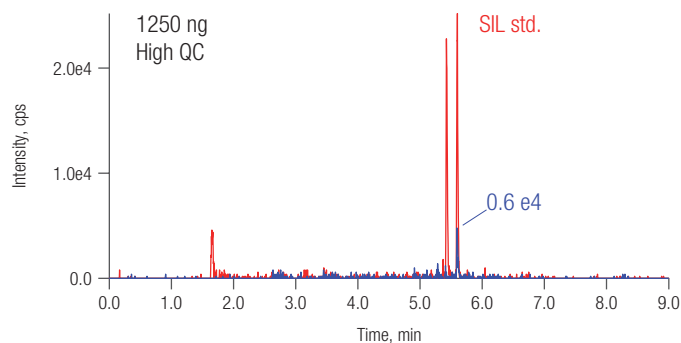
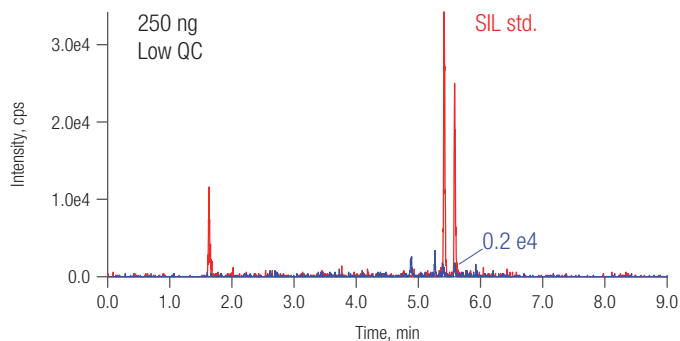


Standard curve (n = 1)			Quality controls (n = 4)		
Actual conc (ng/mL)	Accuracy (%)	Calc value (ng/mL)	Actual conc (ng/mL)	CV (%)	Accuracy (%)
125	93	116.5			
250	107	266.3	250	11.5	90.2
500	106	531.1			
1250	100	1247	1250	7.4	99.1
2500	90	2251			
5000	104	2251			

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:

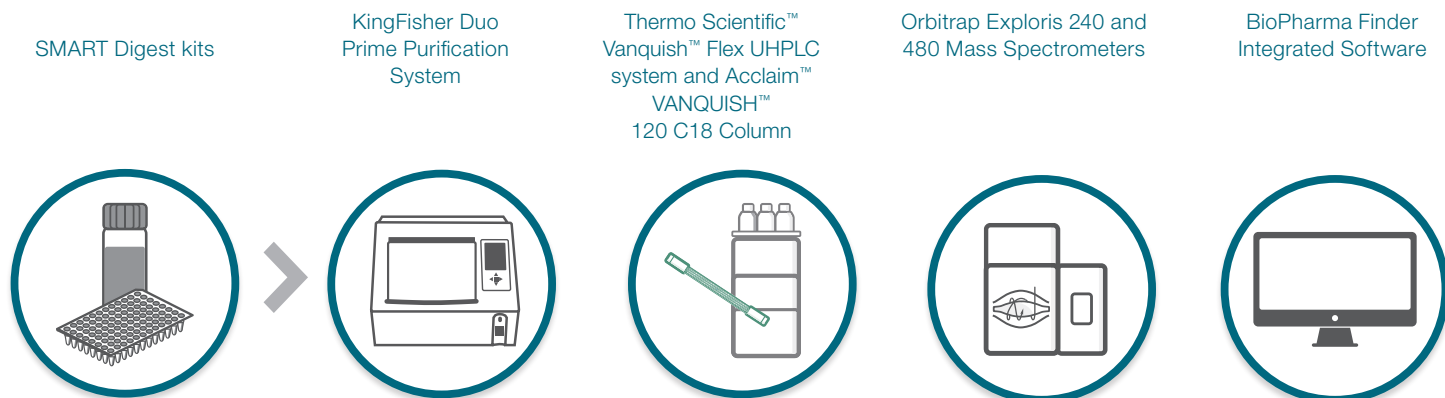
- Significantly faster
- Easier to use
- Highly reproducible
- Sensitive
- Compatible with automation



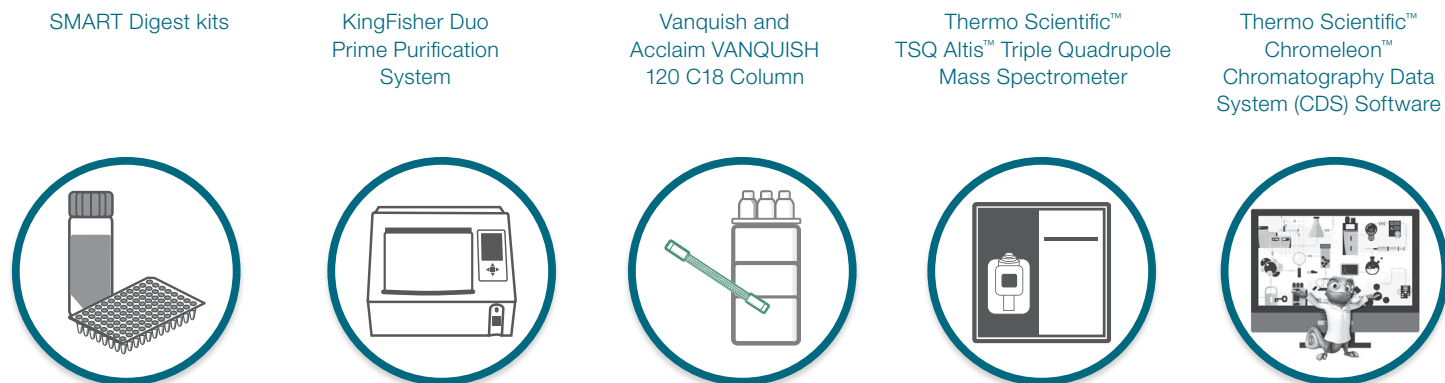
# Peptide mapping and quantitation

Amazingly fast, simple, reproducible, and sensitive protein digestion for peptide mapping and combined immunoaffinity capture and digestion for peptide quantitation, giving you higher confidence in your chromatography and liquid chromatography-mass spectrometry (LC-MS) results.

## Peptide mapping and MAM workflow



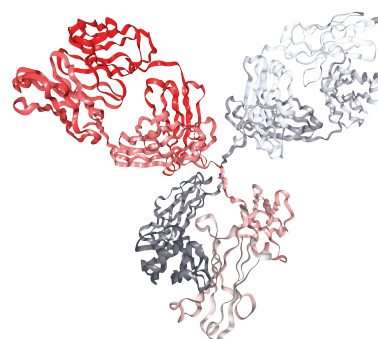
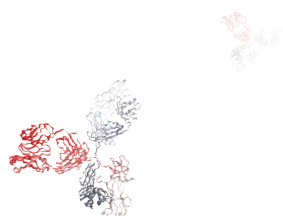
## Peptide quantitation workflow



# 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 $\mu$ /collection plate	60109-103
SMART Digest Trypsin Kit, bulk resin option with SOLA $\mu$ /collection plate	60109-103-B
SMART Digest Trypsin Kit, magnetic bulk resin option with SOLA $\mu$ /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 $\mu$ /collection plate	60109-106
SMART Digest Chymotrypsin Kit, bulk resin option with SOLA $\mu$ /collection plate	60109-106-B
SMART Digest Chymotrypsin Kit, magnetic bulk resin option with SOLA $\mu$ /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 $\mu$ /collection plate	60109-109
SMART Digest Proteinase K Kit, bulk resin option with SOLA $\mu$ /collection plate	60109-109-B
SMART Digest Proteinase K Kit, magnetic bulk resin option with SOLA $\mu$ /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 $\mu$ collection plate	60109-112



## SMART Digest low pH kits for MAM

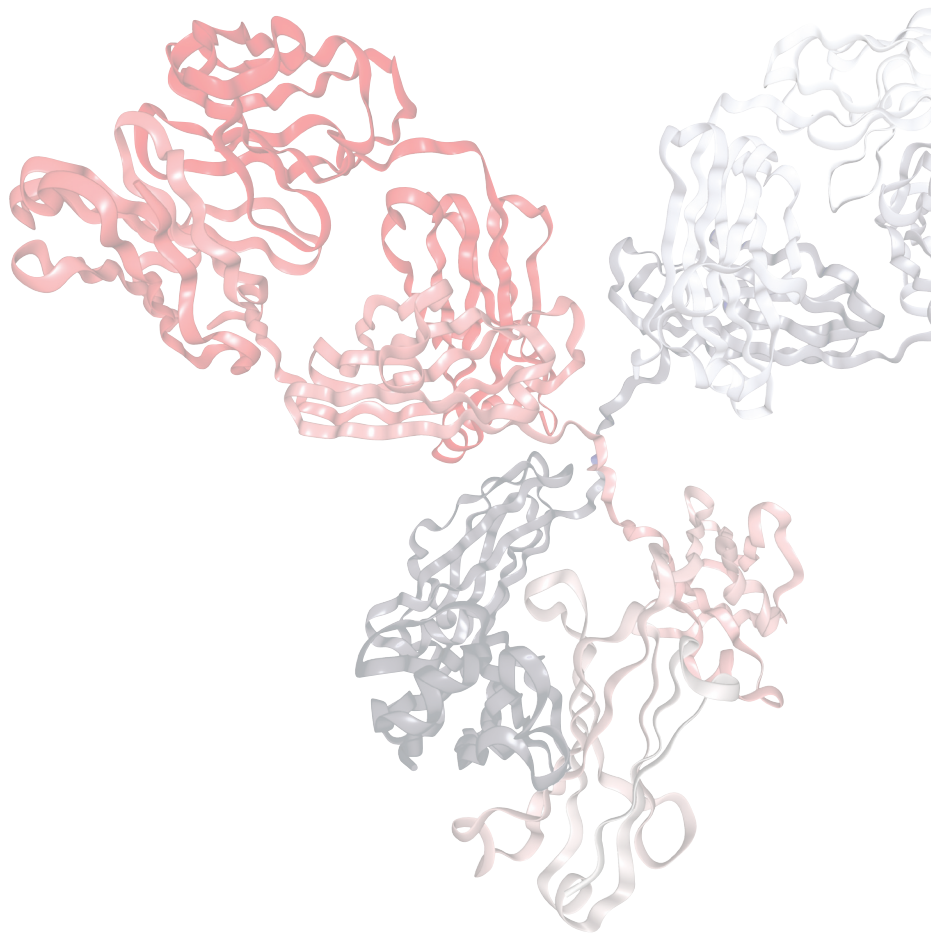
Description	Part number
SMART Digest low pH Kit Trypsin	60109-101-LPH
SMART Digest low pH Kit Trypsin magnetic beads	60109-101-MB-LPH
SMART Digest KIT Chymotrypsin magnetic bulk low pH buffer	60109-104-MB-LPH
SMART Digest low pH Kit Trypsin magnetic beads	60109-101-MB-LPH
SMART Digest Kit Chymotrypsin 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 $\mu$ SPE and collection plate	60110-102
SMART Digest IA Kit, Streptavidin magnetic with SOLA $\mu$ 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 $\mu$ SPE and collection plate	60111-102
SMART Digest IA Kit, Protein A magnetic with SOLA $\mu$ 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 $\mu$ SPE and collection plate	60112-102
SMART Digest IA Kit, Protein G magnetic with SOLA $\mu$ 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 $\mu$ collection plate, soluble Trypsin	60114-102
SMART Digest Bulk Protein A Fractionation Kit, magnetic, with SOLA $\mu$ 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 $\mu$ collection plate, soluble Trypsin	60115-102
SMART Digest Bulk Fractionation Kit, Protein G, magnetic, with SOLA $\mu$ 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 $\mu$ 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 $\mu$ ™ HRP SPE Plate	60209-001
KingFisher Duo Prime Purification System	5400110
Thermo Scientific™ KingFisher™ Flex Purification System	5400630



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