

Thermo Scientific
SMART Digest Kit Technical Guide

Facilitating
perfect digestion

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SMART Digest delivers simple, robust, reproducible and fast digestion of proteins

The Thermo Scientific™ SMART Digest™ kits are designed for biopharmaceutical and proteomic applications that require highly reproducible, sensitive and fast analyses, often in high throughput workflows. They achieve this due to the optimized heat stable immobilized trypsin design.

Challenges for biopharmaceutical and protein research laboratories

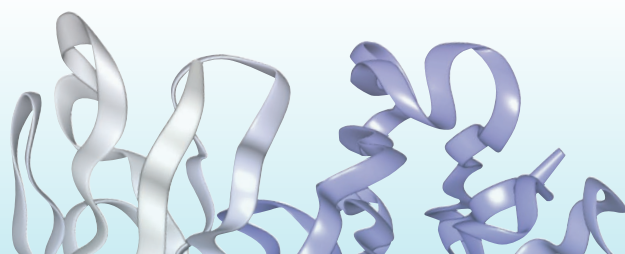
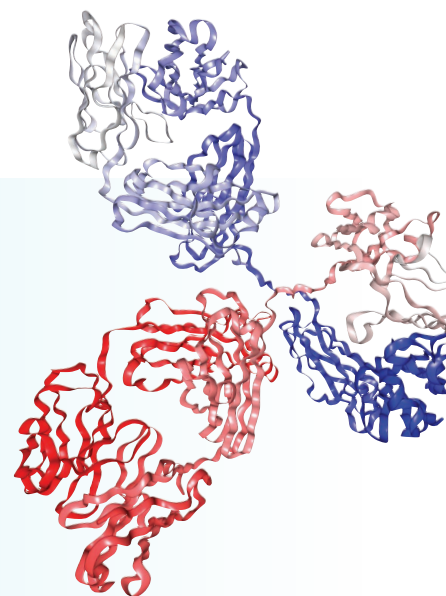
The modern biopharmaceutical and protein research laboratory is tasked with providing high quality analytical results, often in high-throughput, regulated environments. One of the key areas which affects these requirements is sample preparation. Current technologies employed are subject to high levels of irreproducibility, poor sensitivity, and protracted methodologies that are not amenable to automation and often require 24 hours to achieve full digestion.

In order to meet the industry demands, analytical workflows need to provide:

- High reproducibility
- High sensitivity
- High levels of data quality
- Compatibility with automation

The SMART Digest kit is simple to implement and satisfies the analytical workflow demands. It offers significant benefits over existing conventional in-solution digestion protocols.

- Significantly faster than in-solution digestion
- 3-step sample preparation protocol
- Higher throughput
- Ability to automate
- More reproducible than in-solution digestion
- Higher sensitivity



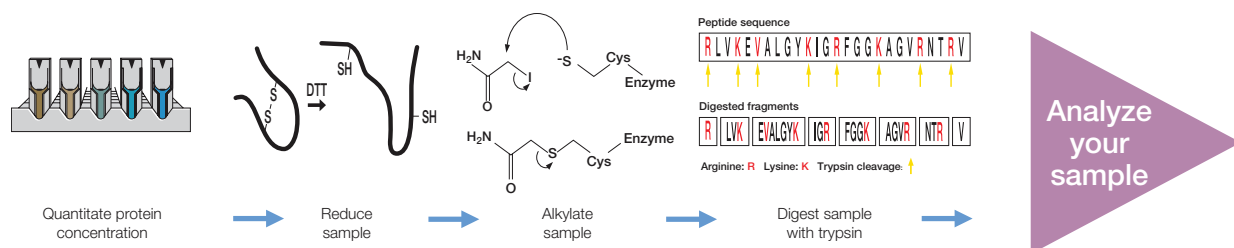
Increased workflow efficiency and simplicity of use with the SMART Digest Kit

It provides a simple to implement sample preparation process, which not only mitigates against errors, but also significantly reduces sample preparation time.

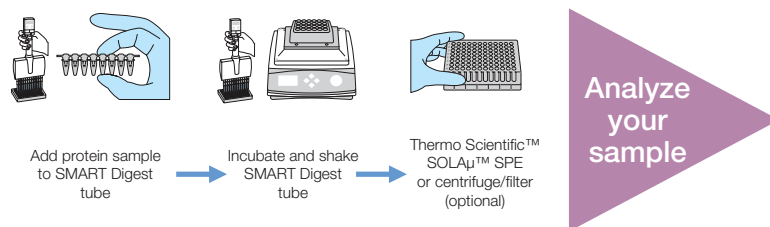
Current sample preparation protocols for the digestion of proteins are multifaceted and laborious. Without a single source solution, errors are far more likely to occur. Therefore, the overall protocol is subject to irreproducibility and significant sample processing time.

The SMART Digest kit overcomes these barriers by providing a 3-step, 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

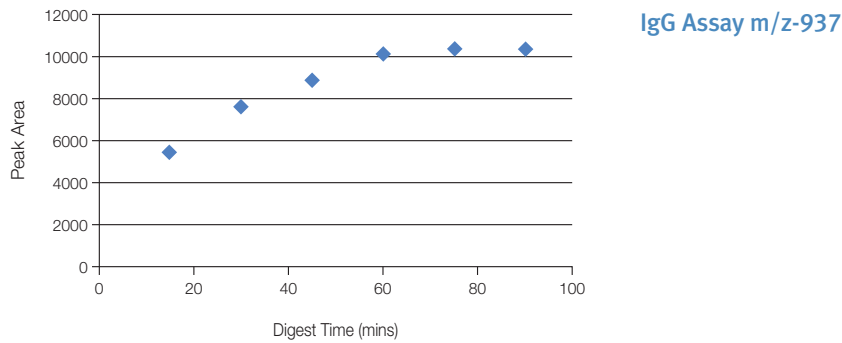


SMART Digest



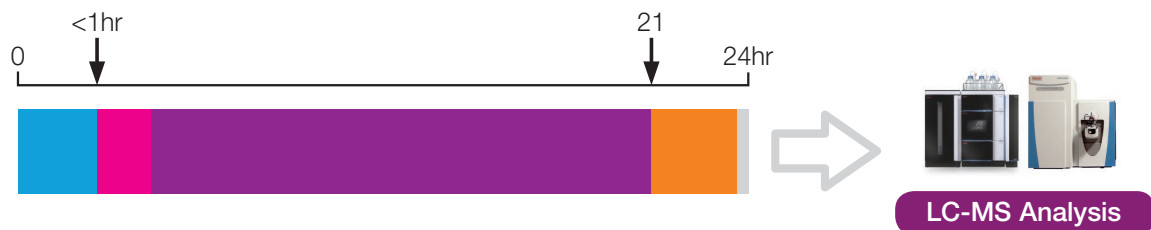
Full digestion is achieved in 60 minutes

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, dependant on sample complexity, to achieve full digestion as can be seen in the IgG example below.



SMART Digest vs conventional in-solution digest protocol

In-solution digest



SMART Digest

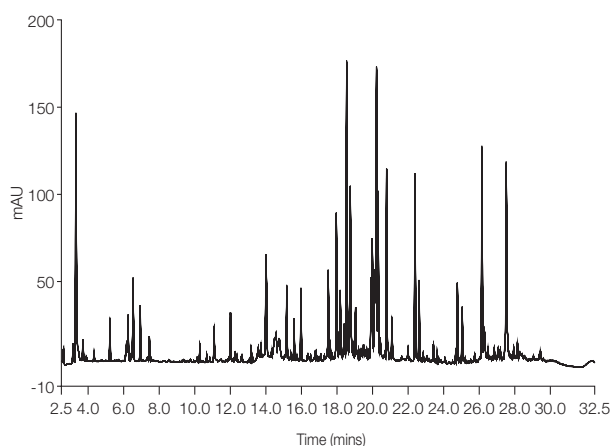


- Sample preparation
- Digestion
- Spin down
- Protein assay
- SPE + dry/reconstitute

Reproducibility

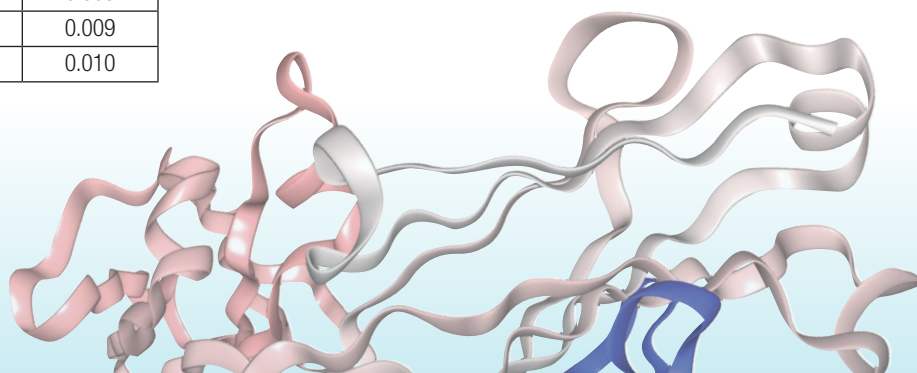
The SMART Digest kit provides significant improvements in reproducibility over existing protocols, which results in fewer sample failures, higher throughput and the ability to more easily interrogate data.

The following chromatogram shows 13 overlaid traces of mouse mAb peptides, extracted from human plasma and processed with the SMART Digest kit. Excellent reproducibility of <0.082 %RSD is achieved, as indicated in the table below.



Instrumentation:	Thermo Scientific™ Vanquish™ UHPLC System	
Column:	Thermo Scientific™ Acclaim™ RSLC 120, C18, 2.2 μm Analytical (2.1 × 250 mm) (P/N 074812)	
Mobile phase A:	0.05% TFA in water	
Mobile phase B:	0.04% TFA in 8/2 acetonitrile/water (v/v)	
LC Gradient:	Time (min)	%A %B
	0.0	96 4
	30	50 50
	31	10 90
	35	10 90
	36	96 4
	45	96 4
Flow Rate:	0.40 mL/min	
Inj. Volume:	5 μL	
Temp.:	80 °C	
Detection:	214 nm, data collection rate 20 Hz, Response Time 0.2 sec	
Data Processing:	Thermo Scientific™ Dionex™ Chromleon™ 7.2 Chromatography Data System	

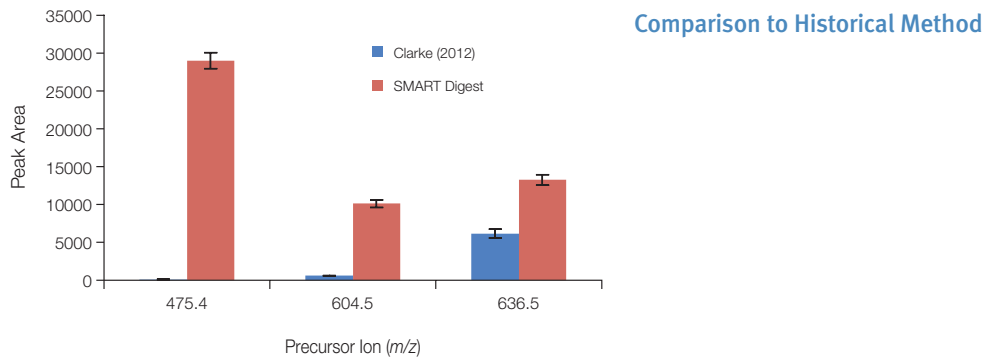
Peak #	RT (min)	RT-RSD (%)
3	3.315	0.082
9	5.231	0.065
14	6.532	0.017
15	6.937	0.023
19	10.290	0.021
23	12.013	0.012
31	14.011	0.013
39	15.177	0.012
42	15.589	0.010
51	17.511	0.007
55	17.969	0.011
61	18.546	0.010
83	20.798	0.010
85	21.095	0.012
87	22.386	0.009
96	24.774	0.012
103	26.155	0.009
106	26.155	0.009
109	27.529	0.010



Sensitivity/Data Quality

The SMART Digest kit provides higher sequence coverage due to the removal of the reduction and alkylation steps and absence of trypsin. This reduces the number of chemicals and presence of trypsin in the final sample, as well as reducing post translational modifications, resulting in lower suppression of signal during LC-MS analysis.

The mass spectrometry (MS) peak area response comparison below shows how the thyroglobulin from human plasma, digested using the SMART Digest kit, provides higher levels of sensitivity compared to a standard in-solution digest*. In addition to this the SMART Digest protocol took 3.5 hours compared to 20 hours for the in-solution protocol.



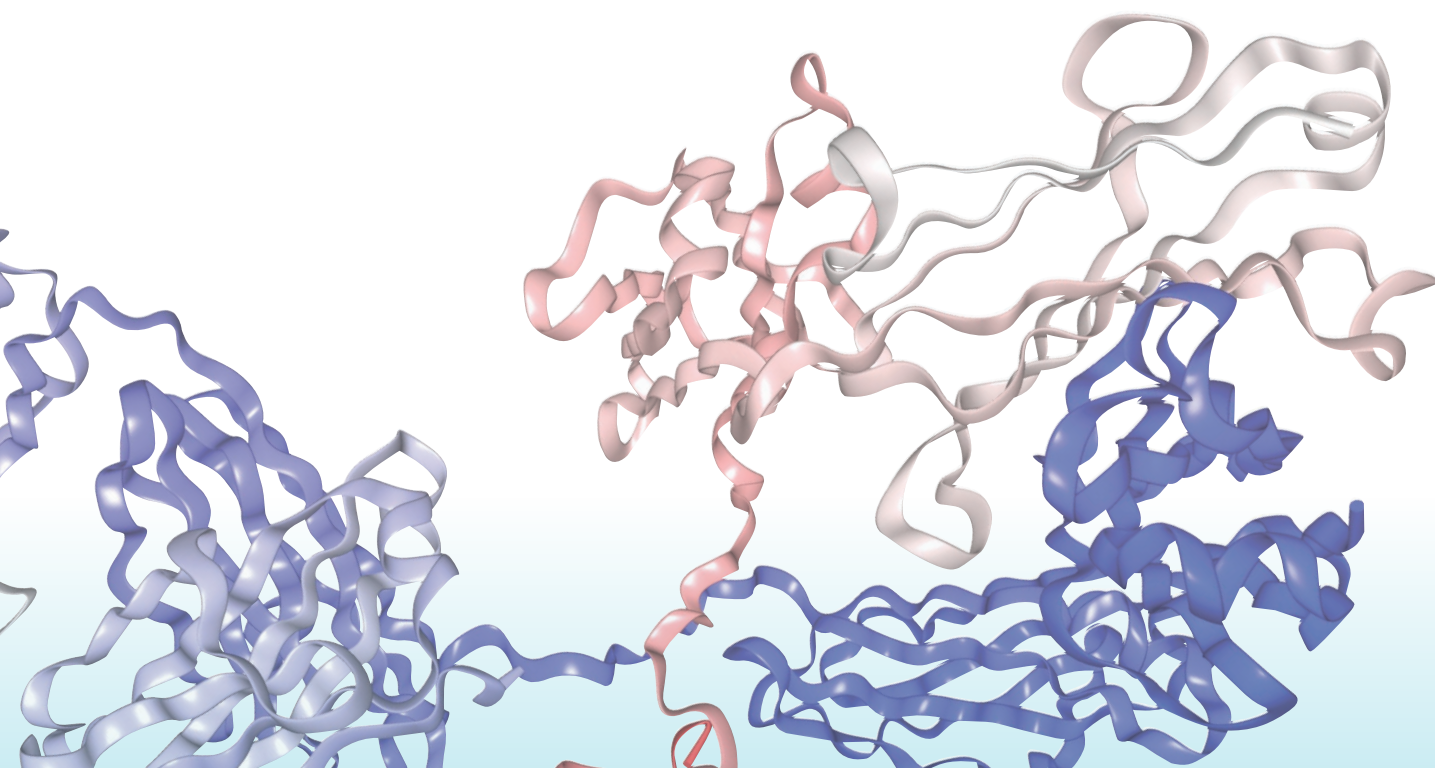
*Clarke, N. J., et al.; *J. Inv. Med.* **2012**, *60*, 1157-1163

Ordering Information

Description	Part Number
SMART Digest kit	60109-101
SMART Digest kit with 96 well filter plate	60109-102
SMART Digest kit with SOLA μ HRP SPE plate	60109-103

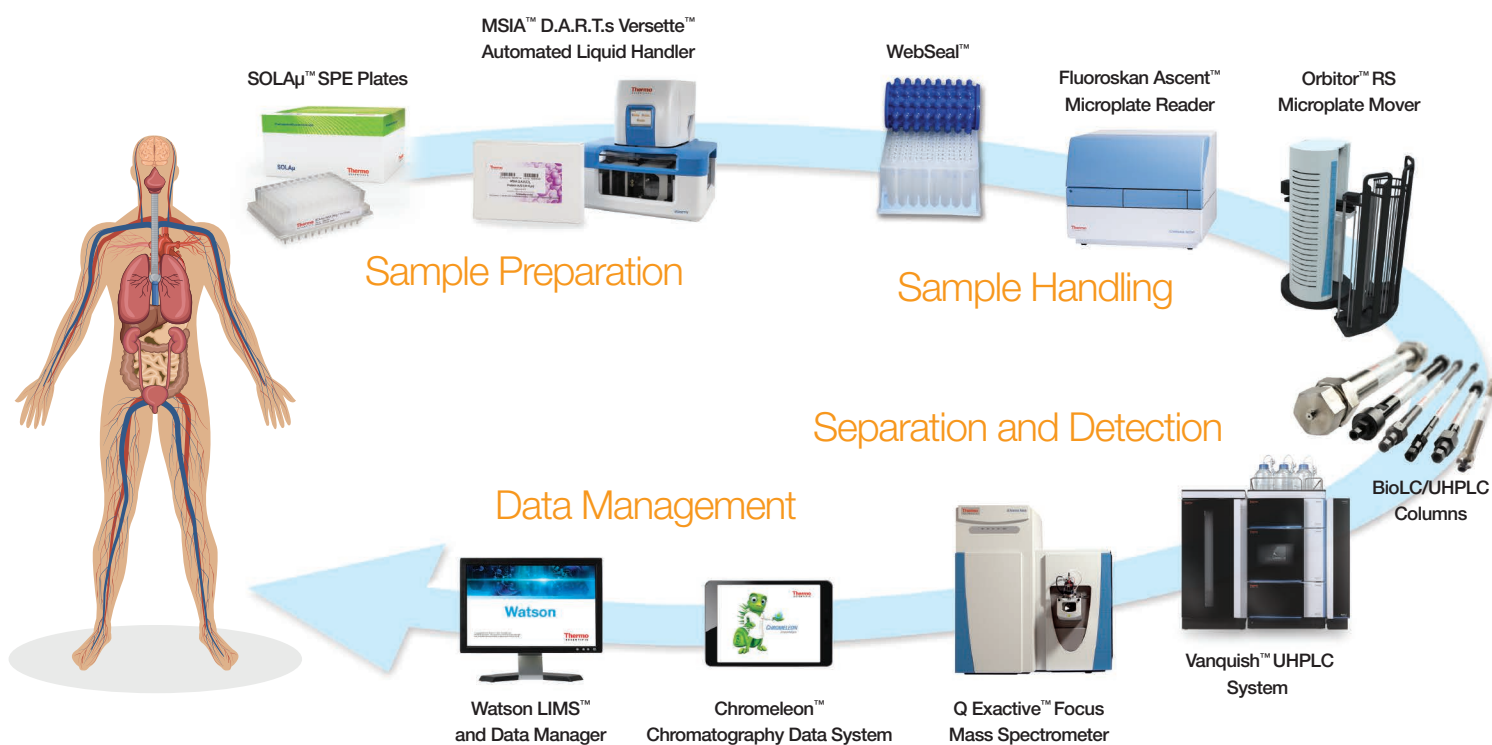
Complementary Products

Description	Part Number
Thermo Scientific™ 96 well vacuum manifold	60103-351
Thermo Scientific™ vacuum pump (NA)	60104-243
Thermo Scientific™ vacuum pump (EU)	60104-241
SOLA μ HRP SPE plate	60209-001



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