thermo scientific

Questions Answers

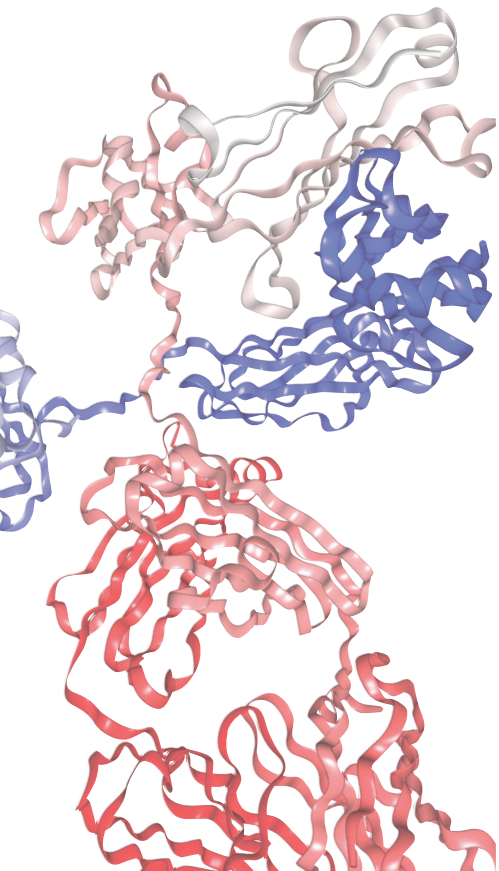
SMART Digest Kit
Facilitating perfect digestion

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 often require 24 hours to achieve full digestion.

The Thermo Scientific™ SMART Digest™ kits remove these issues by providing a digestion solution which is:

- Fast
- Simple
- Highly reproducible

Following are some frequently asked questions relating to how the technology works and how it can be implemented.



ThermoFisher SCIENTIFIC

Questions Answers

Question: What heater shaker device should I use?

Answer: The PCR format is key to sample reproducibility; shaking is a necessity to avoid any diffusion limitations. A heater shaker device with the following features is required:

- PCR heating block
- Heated lid
- Shaking

Question: Can I use my standard PCR instrument?

Answer: Unfortunately, no. Shaking is a necessity to avoid any diffusion limitations.

Question: What kind of samples have you worked with?

Answer: To date we have successful applications in mouse, monkey, beagle and human plasmas. We also have successful applications in cell lysates, urine and cerebral spinal fluid.

Question: What is the maximum volume of materials that can be digested?

Answer: 200 µL.

Question: What is the minimum volume of materials that can be digested?

Answer: 50 µL.

Question: What amount of materials can be digested?

Answer: Up to 50 µL of plasma (approx. 3.5 mg) and as little as 200 pg.

Question: How much trypsin is there in each well?

Answer: Each well contains 14 µg of immobilized trypsin.

Question: Can you vary the amount of trypsin used depending on protein load?

Answer: One of the benefits of using immobilized trypsin is that there is reduced autolytic activity. As such, there is no need to vary the amount trypsin used for any given sample.

Question: What is a typical digestion time?

Answer: All proteins vary with regards to digestion; adjust temperature and incubation time accordingly. A recommend strategy for screening digestion time is outlined below.

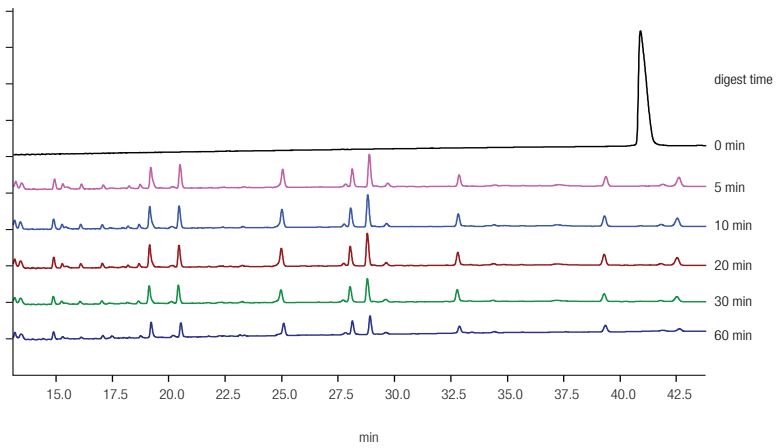
1. Create a method in your heater/shaker–set temperature to 70 ºC and RPM to1400.
2. Allow the temperature to reach equilibrium for at least 5 minutes.
3. Prepare 8 identical samples using a relatively high known concentration of native analyte in the matrix of operation (dilute them to 50 µL with ultrapure water, if necessary) and add to individual SMART Digest wells.
4. Add 150 µL of SMART Digest buffer to each well and cap.
5. Place all wells firmly into the preheated Heater Shaker.
6. Periodically (e.g. every 5 to 15 minutes) remove a sample from the strip.
7. Centrifuge, filter or perform an SPE process with a SOLAµ HRP plate (60209-001) then analyze the samples to determine the extent of digestion (*see diagram and table below*).
8. Once the intact protein peak has disappeared, digestion is complete and the corresponding digestion time can be used for subsequent analyses (see example of carbonic anhydrase below).

Trypsin digests within minutes

Recommended digestion starting conditions for known proteins*	
Protein	**Digest Time (min)**
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 µL plasma	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

* 200 µL protein solution (100 µg/mL);
IgG in plasma: 17.5 µg/mL
Temperature: 70 °C

Carbonic Anhydrase, 29 KDa



This diagram shows a time course experiment for the digestion of carbonic anhydrase.

By removing consecutive samples and monitoring the disappearances of the intact peak the optimum digestion time can be determined.

In this case full digestion is complete in **5 minutes.**

Question: Do I have to use the SMART Digest buffer?

Answer: The SMART Digest buffer was optimized for maximum trypsin activity at elevated temperatures. Other buffers can be used, but their use may negatively impact trypsin activity. If your application requires the use of an alternative buffer, digestion time and temperature should be optimized accordingly.

Question: Are there salts in the SMART Digest buffer?

Answer: The SMART Digest buffer contains about 0.5M salts. These salts greatly assist in achieving rapid digestion at high temperatures. Desalting through the use of valve switching, or the use of Thermo Scientific™ SOLAμ™ SPE cleanup is advised.

Question: What is the pH of the SMART Digest buffer?

Answer: The pH is approximately 7.2.

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

Answer: The SMART Digest kits were engineered to be thermally stable. When operated at high temperatures (e.g. 70 ºC), denaturation and digestion occurs 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. Denaturants and reducing reagents can negatively impact digestion using the SMART Digest kits.

Question: Will disulfide bonds scramble during digestion?

Answer: If there are free cysteines, it is possible for disulfides to scramble before, during or after digestion. We would therefore recommend performing alkylation prior to digestion.

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

Answer: 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.

Question: Can I use surfactants with SMART Digest kits?

Answer: Many surfactants negatively impact not only digestion, but LC-MS performance as well. Of the surfactants we have screened, octylglucoside is the only surfactant that does not negatively impact trypsin activity. It is not charged, so does not impact MS ionization and exists as one molecular weight, it therefore does not result in multiple background peaks.

Question: Is the trypsin mutated to be heat stable? Would this affect my sample?

Answer: During the immobilization process the trypsin is chemically modified in such a way that it is chemically stabilized while maintaining it's specificity. The selectivity of the cleavage site is not affected.

Question: What helps mitigate against trypsin self-digestion (autolysis)?

Answer: The immobilization of trypsin prevents it from attacking and digesting itself, contrary to what happens in an in-solution digestion.

Question: Is complete sample digestion achieved?

Answer: Yes, extensive studies have shown that complete sample digestion is achieved in as little as 5 minutes for simple mono-protein samples, to 3.5 hours for complex matrices such as plasma.

Question: What is the resin made of to which the trypsin is coupled in the SMART Digest kit?

Answer: 10 µm PS-DVB core made hydrophilic with a two-tailed coating.

Question: Why do I need to perform reduction and alkylation post digestion?

Answer: The reducing agent lowers digestion efficiency and adds extra steps unless you are specifically looking for disulphides.

Question: Is it compatible with isobaric tagging e.g. SILAC, ITRAQ etc….?

Answer: Yes, perform post digestion.

Question: Is the SMART Digest kit compatible with gels?

Answer: No, as the beads are unable to penetrate gels and start digestion.

Question: Can you use the SMART Digest kit with Lys-C or other enzymes?

Answer: Yes, perform post digestion.

Question: Why do you not need to use urea to unfold the protein?

Answer: The reason urea is needed as a first step in an in-solution protocol is to disrupt the sample and partially unfold the proteins. The proteins need to be partially unfolded so that the trypsin enzyme can have better access to the internal amino acid chain, not just the surface of the protein of interest.

The reason the SMART Digest kit doesn't need urea is that it uses heat to unfold the protein.

Question: Why don't we need to quantitate the protein?

Answer: The SMART Digest kit contains an excess of enzyme capable of digesting between 200 pg and 3.5 mg of protein. As most samples will fall in this range it is not necessary to routinely quantitate protein concentration prior to digestion.

Question: What is the composition of the SMART Digest buffer?

Answer:

Chemical Name	CAS No.	EINECS No.	Kit Component	Weight %
Water	7732-18-5	231-791-2	2	50-95%
Glycerol	56-81-5	200-289-5	2	< 20%
Tris Base	77-86-1	201-064-4	2	< 10%
Tris-HCl	1185-53-1	214-684-5	2	< 10%
Calcium Chloride	10043-52-4	233-140-8	2	< 10%
Sodium Azide	26628-22-8	247-852-1	2	< 0.1%

Question: How much urea can be used with the SMART Digest kit?

Answer: The SMART Digest kit is not affected by concentrations of up to 0.5 M of urea. If the concentration is higher than this we recommend that the sample is diluted to <0.5 M of urea, prior to beginning digestion using the SMART Digest kit.

Question: How compatible is the SMART Digest kit with detergents e.g. CHAPS, OGS, TWEEN and RIPA?

Answer:

- CHAPS – Reduction ~ 30% in digest efficiency.
- OGS – no reduction in digestion efficiency.
- TWEEN – no reduction in digestion efficiency.
- RIPA – ~The addition of RIPA, for ribonuclease A digestion, results in a concentration-dependent effect, where initial enzyme inhibition is overcome by improved substrate solubilization at higher concentrations only. 20% reduction in digestion efficiency.

thermo scientific

The **SMART Digest kit** is simple to implement and satisfies the analytical workflow demands of the biopharmaceutical industry.

It offers significant benefits over existing conventional in-solution digest protocols.

- Significantly faster
- Simple protocol
- More reproducible

Ordering Information

Description	Part Number
SMART Digest kit	60109-101
SMART Digest kit with 96 well filter plate	60109-102
SMART Digest kit with SOLAμ 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
SOLAμ HRP SPE plate	60209-001

Find out more at **thermofisher.com/SMARTdigest**

ThermoFisher SCIENTIFIC