thermo scientific

Questions Answers

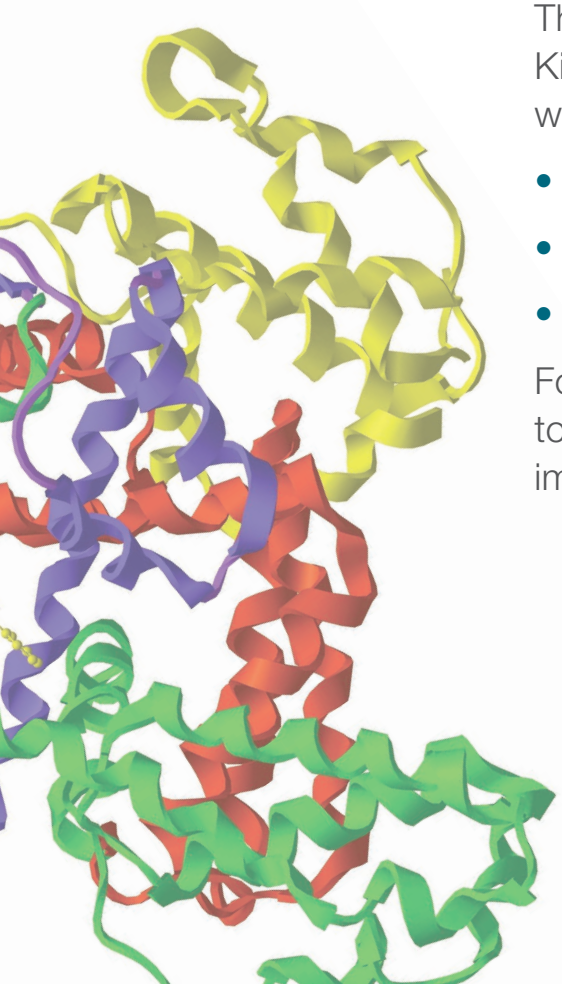
SMART Digest Soluble Trypsin Kit
Smarter protein digestion

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™ Soluble Trypsin Kit removes 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 equipment is required?

Answer: Any heating unit with a heated lid, capable of providing uniform heating at 70 °C may be used.

Question: Is shaking required?

Answer: As long as the substrate is soluble, shaking is not required.

Question: When we use 5 µL of the SMART Digest soluble trypsin kit, how much trypsin is present?

Answer: The enzyme concentration is 1 µg/µL so that would be 5 µg.

Question: What amount of materials can be digested?

Answer: For every 5 µL of the SMART Digest soluble trypsin kit up to 50 µL of plasma (approx. 3.5 mg of protein) is readily digested.

Question: Do I need to vary the amount of trypsin used depending on protein load?

Answer: Generally, there is no requirement to vary the amount of trypsin used for any given sample. Studies have shown that when operating at elevated temperatures with a stable enzyme, the reactions are significantly less concentration dependant than traditional protocols. For samples containing less than 5 µg of total protein, titering the enzyme amount may prevent an excess of enzyme from interfering with the analysis.

Question: What is a typical digestion time?

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

1. Create a method in your heating unit setting the desired operating temperature (70 °C recommended). Start this method and allow the temperature to reach equilibrium for at least 5 minutes before adding samples.

2. Prepare eight identical samples using a relatively high known concentration of native analyte in the matrix of operation, diluting them to 50 µL each with ultrapure water, if necessary.

3. Add each sample along with 150 µL of SMART Digest buffer and 5 µL of SMART Digest soluble enzyme to each of the eight wells.

4. Place all samples firmly into the preheated unit.

5. Periodically (e.g. every 15 minutes) remove a sample and quench the reaction using an equivalent volume of 1% trifluoroacetic acid, or 1% formic acid.

6. Following incubation analyze the samples to determine the extent of digestion.

7. Once the intact protein peak has disappeared, digestion is complete and the corresponding digestion time can be used for subsequent analyses.

DO NOT reduce and alkylate sample prior to digestion. Our findings indicate that the chemicals used for denaturation, reduction and alkylation may negatively impact the activity of the enzyme and the solubility of the protein sample. If the peptides of interest require reduction prior to analysis it is recommended that these steps be performed post digestion.

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 0.5 M salts. These salts greatly assist in achieving rapid digestion at high temperatures. Desalting through the use of valve switching is highly advised, although the use of SPE cleanup has also been successful.

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

Answer: The pH of the digestion buffer as received is approximately 8. When used at 70 °C, the pH of the buffer is approximately 7. Operation at this lower pH helps to prevent the formation of artificial modifications.

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

Answer: The SMART Digest soluble trypsin kit was engineered to be thermally stable. When operated at high temperatures (e.g. 70 °C), denaturation and digestion happen 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 such, for many identification workflows it is recommended that you perform reduction and alkylation after digestion. In our experience denaturants and reducing reagents negatively impact digestion when using the SMART Digest soluble trypsin kits.

Question: Will disulfide bonds scramble during digestion?

Answer: See the publication, 'Thiol-Disulfide Exchange in Human Growth Hormone'[1] for details. No disulfide bond rearrangement was observed. However, free cysteines could potentially lead to scrambling. As needed, alkylate free cysteines to prevent disulfide bond scrambling.

Question: Does digestion at high temperatures using the SMART Digest soluble trypsin kit result in an increase in chemical modifications?

Answer: Thus far studies have shown that by accelerating the reaction and minimizing the reaction time, fewer chemical modifications are observed in comparison to protocols utilizing elongated digestion times at lower temperatures. For more details refer to the application note titled 'SMART Digest Compared to Classic In-Solution Digestion of Rituximab for In-Depth Peptide Mapping Characterization (AN72141).[2]

Question: Can I use surfactants with the SMART Digest soluble trypsin kit?

Answer: The kit is compatible with many surfactants including OGS, CHAPS, Nonidet, and NP-40.

Question: Can I use chaotropes with the SMART Digest soluble trypsin kit?

Answer: The kit is compatible with up to 1M urea, but incompatible with guanidine at most concentrations.

Question: What is the best way to automate my workflow?

Answer: The best way to automate the SMART Digest soluble trypsin kit is to use deepwell plates with Sepra Seals, or PCR plates with iron on seals in combination with an automated liquid handling platform. Any heating unit with a heated lid, capable of providing uniform heating at 70 °C may be used.

Question: Can this kit be used with FASP?

Answer: Yes, the following protocol has been developed and can be optimized as needed:

- Using a fill volume of water rinse a Fisher Scientific™ Vivacon 500, 30kDa MWCO filter (Part Number 14558347) to remove trace amounts of glycerin. Decant the filtrate and collection vessel. The filter is now ready for use. If you do not want to use the Vivacon filter immediately then store the pre-rinsed device in the refrigerator, with water or buffer covering the membrane surface. Do not allow the membrane to dry out.
- Dilute sample water to a total volume of 150 µL on the filter.
- Centrifuge 14,000 RPM for 15 minutes and discard the flow-through.
- Add 150 µL water.
- Centrifuge 14,000 RPM for 15 minutes and discard the flow-through.
- Add 200 µL of SMART Digest buffer.
- Change the collection vial and add 5 µL of the SMART Digest soluble enzyme to the filter.
- Parafilm the filters to prevent evaporation of sample during digestion. Incubate the units in a heated bath at 70 ºC for 60–90 minutes.
- Optional: As needed add DTT (3 µL of 50 mM DTT), heat at 37 ºC and incubate for 30 minutes. Alkylate as needed (using 3 µL of 150 mM alkylating reagent).
- Following incubation, add 2 µL of 1% trifluoroacetic acid to the filter. Mix for 1 minute.
- Centrifuge the filter units at 14,000 RPM for 10 min.
- As needed, measure protein concentration using a Thermo Scientific™ NanoDrop Spectrophotometer, or similar procedure.

References

1. Pharm Res (2016) 33:1370–1382 DOI 10.1007/s11095-016-1879-3
2. http://tools.thermofisher.com/content/sfs/brochures/AN-1159-SP-SMART-Digest-Peptides-AN72141-EN.pdf

thermoscientific

The **SMART Digest soluble trypsin 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
- High sensitivity
- Simple protocol
- Amenable to automation
- More reproducible

Ordering Information

Description	Part Number
SMART Digest Soluble Trypsin Kit and Collection Plate	60113-101

Complementary Products

Description	Part Number
Thermo Scientific™ 96 well Vacuum Manifold	60103-351
Vacuum Pump (NA)	60104-243
Vacuum Pump (EU)	60104-241
SOLAμ HRP SPE Plate	60209-001
SMART Digest Filter Plate	60109-102-F
KingFisher Duo Prime Purification System	5400110
KingFisher Flex Purification System	5400630

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

ThermoFisher SCIENTIFIC