

SMART Digest Kit

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SMART Digest Kit

Delivering simple, robust, reproducible and fast digestion of proteins

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

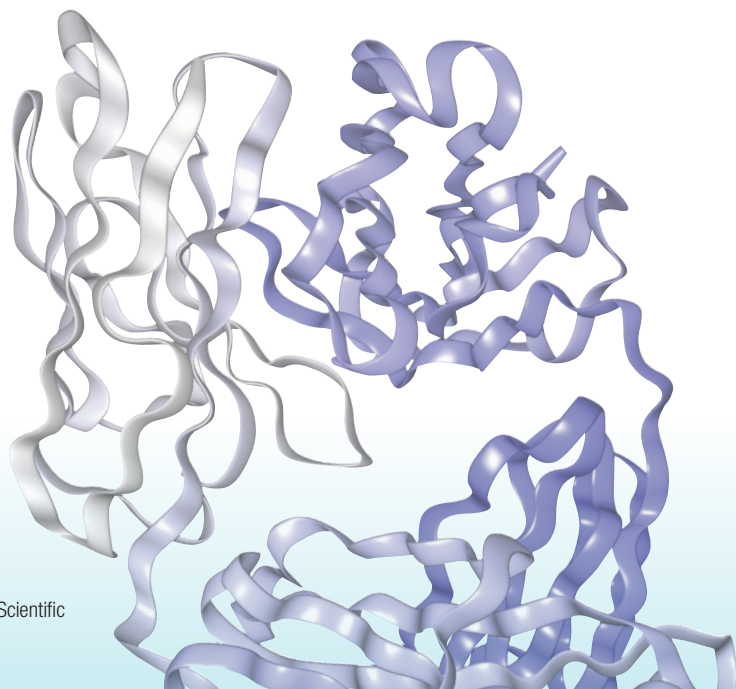
SMART Digest Kit contents:

- 96 SMART Digest tubes (allowing for automation)
- SMART Digest buffer (contains CaCl_2 —not compatible with carbonate or phosphate buffers)
- Filter plate (hydrophilic PVDF), optional
- Thermo Scientific™ SOLA μ ™ HRP SPE plate, optional
- Collection plate (high purity polypropylene)*

**If pure protein or peptide analyte is known to adsorb to plastic materials such as polypropylene, 0.05% BSA (0.5 mg/mL BSA) can be added to the digest buffer.*

Equipment required for operation:

- 96 well vacuum manifold (P/N 60103-351)
- Thermo Scientific™ vacuum pump NA (P/N 60104-243)
- Thermo Scientific™ vacuum pump EU (P/N 60104-241)
- Heater/shaker equipped with PCR block and heated lid



Protein Digestion Procedure

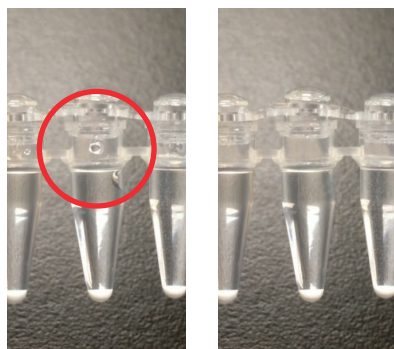
- Sample preparation—if using less than 50 μL of sample, dilute sample to 50 μL using ultrapure water, then dilute fourfold in SMART Digest buffer (final volume of 200 μL per sample).
- Create a method in your heater/shaker system, setting the desired operating temperature to 70 °C and the RPM to 1400. Start this method and allow the temperature to reach equilibrium for at least 5 minutes before adding samples.

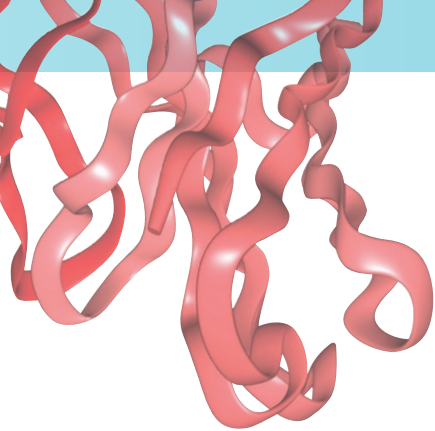
Recommended digestion starting conditions for known proteins*	
Protein	Digest Time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 μL plasma	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

*200 μL protein solutions were used for all samples. A concentration of 100 $\mu\text{g}/\text{mL}$ was used for all samples, except IgG in plasma which used 17.5 mg/mL .

- Set aside the desired number of SMART Digest strips (can be cut into sections).
- Carefully remove the caps from the SMART Digest strips and add samples.
- Recap the samples, making sure all caps are securely fastened.

Improper use can result in evaporation





- Place tubes firmly into the digital shaking drybath and fasten the lid.
- Samples are now ready for filtration or desalting via SPE.
- Place your filter plate on top of your collection plate. After incubation carefully remove the strip caps and transfer samples to the filter plate*. Apply positive pressure or vacuum as needed.
**Optional: Samples can be centrifuged and decanted in place of use of a filter plate. After centrifugation, transfer supernatant samples into collection plate or appropriate sampling holder.*
- The SMART Digest buffer contains salts including CaCl_2 as well as glycerol. It is advised that these are removed prior to analysis by performing an on-line or off-line SPE cleanup: recommended is the SOLA μ HRP SPE Plate (P/N 60209-001).

Operational Specifications

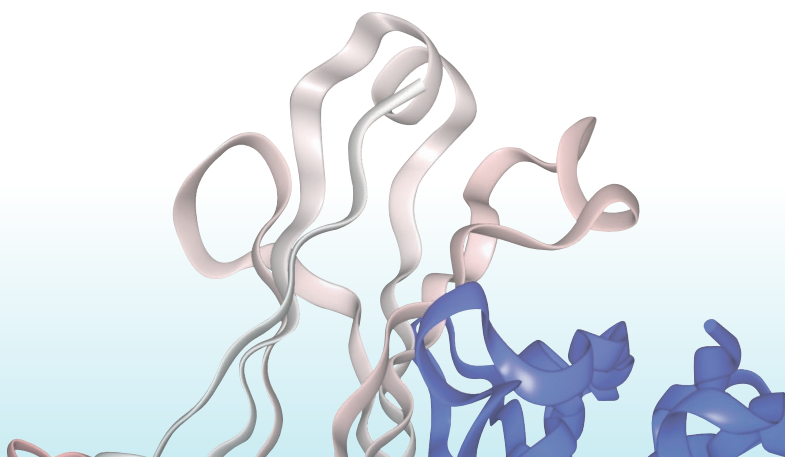
Sample Volume	$\leq 50 \mu\text{L}$
Digestion Buffer	SMART Digest buffer
Operating Temperature	50–70 °C
Storage Temperature	-20 °C

Optimization

All proteins vary with regards to digestion; adjust temperature and incubation time accordingly.

Typically, in order to optimize digestion time, a known relatively high concentration of native analyte in the matrix of operation is placed into each of 8 wells of a SMART Digest strip. Strips can be used as provided, or cut into smaller sections depending on the number of samples to be analyzed. Periodically, a section is removed and the digested materials are desalted by filtration, or SPE prior to analysis.

DO NOT reduce and alkylate sample prior to SMART Digestion. Chemicals used for denaturation, reduction and alkylation negatively impact the activity. If the peptides of interest require reduction prior to analysis it is recommended that these steps be performed post digestion.



Desalting Method with SOLA μ HRP SPE Plate

Equilibrate	200 μ L acetonitrile
Condition	200 μ L 0.1% trifluoroacetic acid
Load	Sample diluted 1:1 with 0.1% trifluoroacetic acid
Wash	500 μ L 0.1% trifluoroacetic acid
Wash	100 μ L 0.1% formic acid
Elute	2 \times 25 μ L 70% acetonitrile in water
Post Extract	Dilute 1:1 with either 0.1% formic acid, or BSA solution in water if there are concerns over non-specific binding

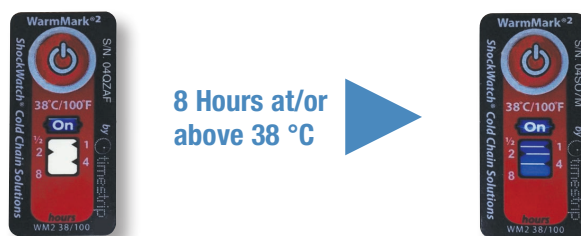
Product Storage

Store SMART Digest strip and resin materials at -20 $^{\circ}$ C and buffer at 4 $^{\circ}$ C.

Each SMART Digest kit will come with a WarmMark² Temperature Indicator. This indicator tracks how long the kit has been at or above 38 $^{\circ}$ C by irreversibly turning from white to blue.

The maximum indicator time is 8 hours, however, should the time indicator reach 2 hours contact www.thermoscientific.com/chromexpert to determine the shipping conditions experienced and ascertain the functionality of the SMART Digest Kit.

WarmMark² Time/Temperature Indicator

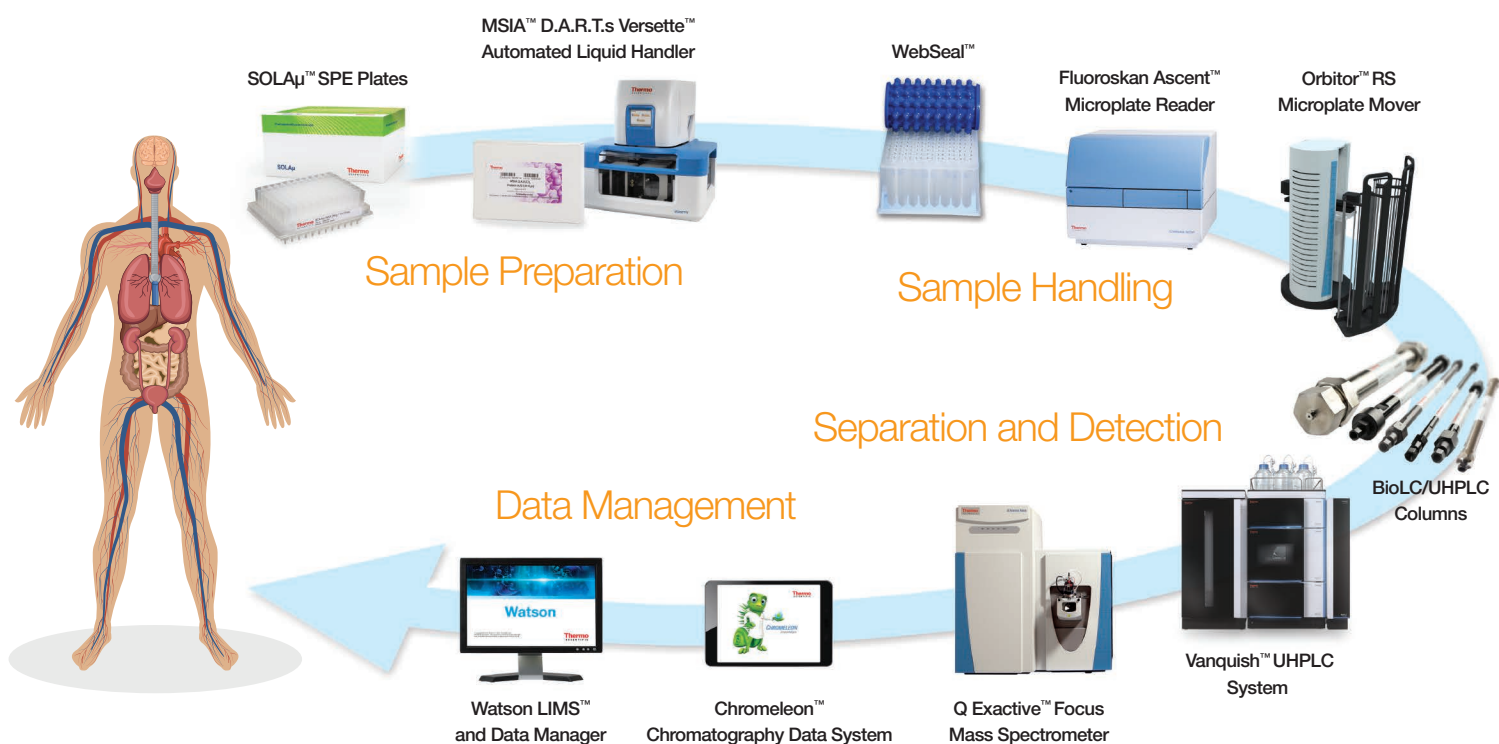


Ordering Information

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

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Technical Support

For advice and support, please visit our website:
www.thermoscientific.com/chromexpert

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