

Frequently Asked Questions (FAQs)

What is the best way to order kits inside of the United States?

Please visit us at www.perfinity.com to order on-line.

Item	Cat. No.
Soluble SMART Digest Kit	700-2001

What equipment is required?

Any heating unit providing uniform heating at 70°C may be used.

What amount of materials can be digested?

For every 5uL of Soluble SMART Digest up to 50uL of plasma (approx. 3.5mg) is readily digested.

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

There is no requirement to vary the amount 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

What is a typical digestion time?

All proteins vary with regards to digestion; adjust temperature & incubation time accordingly. A recommend 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 8 identical samples using a relatively high known concentration of native analyte in the matrix of operation, diluting them to 50µL each with ultrapure water, if necessary.
3. Add each sample along with 150µL of SMART Digest Buffer to each of 8 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% TFA or 1% Formic Acid.

6. Following incubation analyze the samples to determine the extent of digestion.
7. Once the digestion results appear asymptotic, select a digestion time sufficiently within the asymptote to ensure consistent digestion.

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

Do you have other proteases?

At present we offer temperature stable trypsin only. However, if there are additional enzyme you are interested in using, please let us know so that we might expand our product line in a way that best suits your needs.

Do I have to use the SMART Digest Buffer?

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.

Are there salts in the SMART Digest buffer?

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.

What is the pH of the SMART Digest Buffer?

The pH is approximately 7.2.

Do I have to reduce and alkylate my protein?

SMART Digest was engineered to be thermally stable. When operated at high temperatures (e.g. 70°C), denaturation and digestion happen simultaneously. As such, 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 digest using the SMART Digest kits).

Will disulfide bonds scramble during digestion?

If there are free cysteines, it is possible for disulfides to scramble before, during or after digestion. As such, we would recommend performing alkylation prior to digestion.

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

Thus far studies have shown that by accelerating the reaction and minimizing the reaction time, fewer post-translational modifications are observed in comparison to protocols utilizing elongated digestion times at decreased temperatures.

Can I use surfactants with SMART Digest?

Many surfactants are compatible with the Soluble SMART Digest Kit. However, some surfactants deactivate trypsin, leading to diminished returns. If your application requires the use of a detergent, digestion time and temperature should be optimized accordingly.