APPLICATION BRIEF 22016

In-gel digestion using the SMART Digest Trypsin Kit

Authors: CCS Center of Excellence (CoE) Application Scientists, Thermo Fisher Scientific

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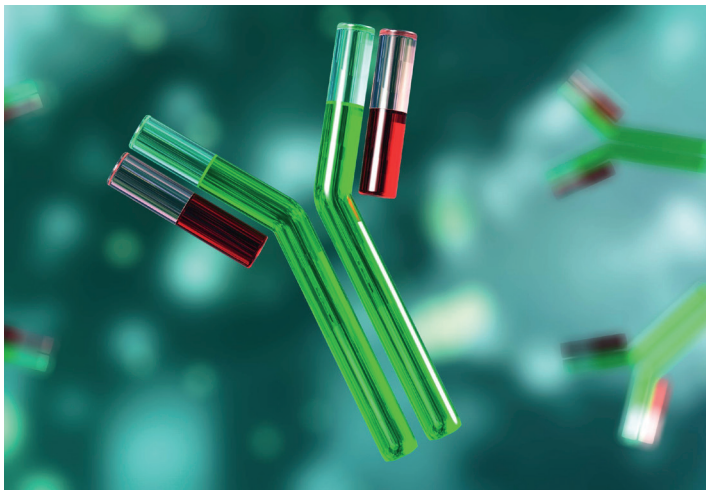

Introduction

In-gel digestion of proteins that have been separated using one-dimensional or two-dimensional gel electrophoresis and visualized using Commassie stains are common and widely available. The recovered peptides can be separated and analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) on instruments such as the Thermo Scientific™ Q Exactive™ Orbitrap mass spectrometer to determine the protein's accession number and description using programs such as the Thermo Scientific™ Proteome Discoverer™ software. To improve lab throughput, efficiency and result reproducibility, the tedious process of making the required buffers and solutions can be replaced with the fast and effective Thermo Scientific™ SMART Digest™ Trypsin kit.

Digestion Protocol

1. **Gel plug preparation**
   a. Using a new scalpel, cleaned with high purity ethanol, excise the bands from the gel. Carefully dice the bands into equally sized small plugs so that each sample will use the same rehydration volume.

   b. Place each plug into a 600 µL low retention microcentrifuge tube.

   c. Cover the plugs with 50/50 acetonitrile/water and destain at 70 ˚C on a thermal mixer until the plug is clear.

   d. Dehydrate the plugs using 100% acetonitrile until the plugs appear solid and white.

2. **In-gel digestion**
   a. Prepare the SMART Digest low salt buffer by adding 50 mL of water followed by vortexing.

   b. Add 10 µL of freshly prepared low salt buffer (Step 2a) and 2 µL of soluble trypsin to each 600 µL low retention microcentrifuge tube containing a dehydrated gel band (prepared in Step 1a-d) to be digested.

c. Make sure each band is rehydrated. If the gel-plugs are still not fully hydrated then add a few extra microliters of the low salt buffer to fully hydrate the plug.

d. Incubate on the thermal mixer for one hour at 70 ˚C.

3. **Harvesting the digested peptides**

a. Add 50-100 µL (depends on band size) of 25% acetonitrile with 0.1% formic acid, shake for 10 minutes, and pipette off the washings into a 600 µL low retention microcentrifuge tube.

b. Repeat the process using 50% acetonitrile with 0.1% formic acid.

c. Repeat the process using 75% acetonitrile with 0.1% formic acid.

d. Repeat the process using 10% acetonitrile with 0.1% formic acid.

e. Pool the four collections and dry down using a vacuum concentrator.

f. Reconstitute the peptides using 12 µL of 2% acetonitrile with 0.05% trifluoroacetic acid and transfer into an autosampler vial. Inject 10 µL or less on the instrument if the band is very intense.

Related products

Description	Part number
Acetonitrile, Optima LC-MS Grade, Fisher Chemical	A955-500
Water, Optima LC-MS Grade, Fisher Chemical	W6500
Thermo Scientific™ Pierce™ Formic Acid	PI128905
Trifluoroacetic Acid, Optima™ LC-MS Grade, Fisher Chemical	A116-05AMP
Thermo Scientific™ Snap Cap Low Retention Microcentrifuge Tubes	3446
Thermo Scientific™ Thermal Mixer	13687711

Current versions of product instructions are available at **thermofisher.com/chromexpert**

Learn more about SMART Digest Kits and related products at **thermofisher.com/smartdigest**

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