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# ab270519 FASP Protein Digestion Kit

## A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 44250

View ab270519
FASP Protein Digestion Kit datasheet:
www.abcam.com/ab270519
(use www.abcam.cn/ab270519 for China, or www.abcam.co.jp/ab270519 for Japan)

For efficient digestion of samples for proteome analysis.

This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

FASP Protein Digestion Kit (ab270519) is for researchers who wish to solubilize whole or fractionated protein samples in SDS, digest the protein with trypsin, and analyze the resulting peptides by mass spectrometry. The FASP Protein Digestion Kit provides the necessary columns and buffers to carry out Universal Sample preparation. ab270519 is compatible with a comprehensive range of biological sample types.

## 2. Materials Supplied and Storage

Store at Room temperature. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

| Item | 8 pack | Storage temperature |
|---|---|---|
| Tris-HCl Solution (100 mM) | 1 bottle (20 mL) | Room Temperature |
| Urea | 8 x 0.75 g vials | Room Temperature |
| Iodoacetamide | 8 x 9.8 mg vials | Room Temperature |
| Ammonium Bicarbonate Solution (50 mM) | 1 bottle (20 mL) | Room Temperature |
| Spin Filter (30 kDa MWCO) | 8 | Room Temperature |
| Sodium Chloride Solution (500 mM) | 1 bottle (1 mL) | Room Temperature |
| Collection tube | 16 | Room Temperature |

## 3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Adjustable pipette or multiple-channel pipette
- Microfuge tube
- Trypsin
- Trifluoroacetic acid (TFA)
- Benchtop centrifuge capable of 14,000 *g*
- Incubator set at 37°C
- Vortex

## 4. Technical Considerations

- The FASP Protein Digestion Kit is compatible with whole proteome extracts and other lysates from a wide variety of biological sample types.
- The maximum loading capacity of one FASP Protein Digestion Kit is 0.4 mg protein in up to 30 µL solution.
- Disulfide bonds should be reduced prior to the start of the FASP Protein Digestion protocol for best results. The FASP Protein Digestion Kit is compatible with the common reducing agents dithiothreitol, beta-mercaptoethanol, and tris(2-carboxyethyl) phosphine. If you have used Protein Extraction Kit (ab270054) then proteins have been reduced and do not require further treatment.

## 5. Reagent Preparation

Prepare fresh reagents immediately prior to use.

### 5.1 Urea Sample Solution:

Add 1 mL Tris Hydrochloride Solution provided to one tube of Urea, also provided. Vortex the tube until all the powder dissolves.

### 5.2 10X Iodoacetamide Solution:

Make a 10X Iodoacetamide Solution by adding 100 µL Urea Sample Solution to one tube of Iodoacetamide provided. Mix and dissolve the solution by pipetting it up and down 15 times. Transfer solution to a clean, dry microfuge tube.

### 5.3 Digestion Solution:

Make 75 µL Digestion Solution by dissolving 4 µg trypsin in 75 µL 50 mM Ammonium Bicarbonate Solution provided to a final concentration of 0.05 µg/µL.

## 6. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

### Proteome Extract Digestion protocol:

**6.1** Mix up to 30 µL (0.4 mg) of a protein extract with 200 µL of Urea Sample Solution in the Spin Filter and centrifuge at 14,000 x *g* for 15 minutes.

**6.2** Add 200 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 15 minutes.

**6.3** Discard the flow-through from the collection tube.

**6.4** Add 10 µL 10X Iodoacetamide Solution and 90 µL Urea Sample Solution to the Spin Filter and vortex for 1 minute; incubate without mixing for 20 minute in the dark.

**6.5** Centrifuge the Spin Filter at 14,000 x *g* for 10 minutes.

**6.6** Add 100 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 15 minutes.

**6.7** Repeat this step twice. Discard the flow-through from the collection tube.

**6.8** Add 100 µL of 50 mM Ammonium Bicarbonate Solution provided to the Spin Filter and centrifuge at 14,000 x *g* for 10 minutes. Repeat this step twice.

**6.9** Add 75 µL Digestion Solution (enzyme-to-protein ratio 1:100) and vortex for 1 minute. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.

**6.10** Incubate the Spin Filter in an incubator at 37°C for 4 – 18 hours.

**6.11** Transfer the Spin Filter to a new collection tube.

6.12 Add 40 µL of 50 mM Ammonium Bicarbonate Solution. Centrifuge the Spin Filter at 14,000 x *g* for 10 minutes. Repeat this step once.

6.13 Add 50 µL 0.5 M Sodium Chloride Solution provided and centrifuge the Spin Filter at 14,000 x *g* for 10 minutes.

6.14 Filtrate contains digested proteins. Acidify the filtrate with TFA to the desired pH and desalt.

# Technical Support



**For all technical or commercial enquiries please go to:**

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www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)