expedeon
protein discovery & analysis

FASP™ Protein Digestion Kit
USE AND STORAGE INSTRUCTIONS

INTRODUCTION

Protein Discovery's FASP Protein Digestion Kit 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 as described by Wisniewski, Zoubman, Nagaraj and Mann [i].The FASP Kit is compatible with a comprehensive range of biological sample types. A second protocol, included, provides instructions for digesting molecular weight fractions produced by the Gelfree® 8100 Fractionation System.

STORAGE AND STABILITY

Store FASP Protein Digestion Kit materials at room temperature. Product shelf life is two years.

PROTOCOL 1 USAGE GUIDELINES

PROTEOME EXTRACT DIGESTION

The following usage guidelines refer to the FASP Protein Digestion Kit when it is used in accord with the Proteome Extract Digestion protocol.

- 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 Discovery's UPX™ Universal Protein Extraction Kit or YPX™ Yeast Protein Extraction Kit, then proteins have been reduced and do not require further treatment.

RECOMMENDED PROCEDURE

MATERIALS NEEDED

FASP Protein Digestion Kit
Microfuge tube
Pipettor and Pipette Tips
Trypsin
Trifluoroacetic acid (TFA)
Benchtop centrifuge capable of 14,000 x *g*
Incubator set at 37 ˚C
Vortex

PREPARING UREA SAMPLE SOLUTION

Urea Sample Solution should be prepared fresh prior to digestion.

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

PREPARING 10X IODOACETAMIDE SOLUTION

10X Iodoacetamide Solution should be prepared fresh prior to digestion.

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

PREPARING DIGESTION SOLUTION

Digestion Solution should be prepared fresh prior to digestion.

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

PROTOCOL

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 min.
2. Add 200 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 15 min.
3. Discard the flow-through from the collection tube.
4. Add 10 µL 10X Iodoacetamide Solution and 90 µL Urea Sample Solution to the Spin Filter and vortex for 1 min; incubate without mixing for 20 min in the dark.
5. Centrifuge the Spin Filter at 14,000 x *g* for 10 min.
6. Add 100 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 15 min. Repeat this step twice.
7. Discard the flow-through from the collection tube.
8. Add 100 µL of 50 mM Ammonium Bicarbonate Solution provided with the FASP Kit to the Spin Filter and centrifuge at 14,000 x *g* for 10 min. Repeat this step twice.
9. Add 75 µL Digestion Solution (enzyme-to-protein ratio 1:100) and vortex for 1 min. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.

10. Incubate the Spin Filter in an incubator at 37 °C for 4 – 18 h.
11. Transfer the Spin Filter to a new collection tube.
12. Add 40 µL of 50 mM Ammonium Bicarbonate Solution. Centrifuge the Spin Filter at 14,000 x *g* for 10 min. Repeat this step once.
13. Add 50 µL 0.5 M Sodium Chloride Solution provided with the FASP Kit and centrifuge the Spin Filter at 14,000 x *g* for 10 min.
14. Filtrate contains digested proteins. Acidify the filtrate with TFA to the desired pH and desalt.

PROTOCOL 2 USAGE GUIDELINES

GELFREE 8100 FRACTION DIGESTION

The following usage guidelines refer to the FASP Protein Digestion Kit when it is used in accord with the Gelfree 8100 Fraction Digestion protocol.

RECOMMENDED PROCEDURE

MATERIALS NEEDED

FASP Protein Digestion Kit

Microfuge tube

Pipettor and Pipette Tips

Trypsin

0.1% Formic acid

Benchtop centrifuge capable of 14,000 *g*

Incubator set at 37 °C

Vacuum concentrator

Vortex

PREPARING UREA SAMPLE SOLUTION

Urea Sample Solution should be prepared fresh prior to digestion.

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

PREPARING 10X IODOACETAMIDE SOLUTION

10X Iodoacetamide Solution should be prepared fresh prior to digestion. Make a 10X Iodoacetamide Solution by adding 100 µL Urea Sample Solution to one tube of Iodoacetamide provided with the FASP Kit. Mix and dissolve the solution by pipetting it up and down 15 times. Transfer solution to a clean, dry microfuge tube.

PREPARING DIGESTION SOLUTION

Digestion Solution should be prepared fresh prior to digestion.

- Make 75 µL Digestion Solution by dissolving 1 µg trypsin in 75 µL 50 mM Ammonium Bicarbonate Solution provided with the FASP Kit.

PROTOCOL

1. Add 200 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 5 min.
2. Load 150 µL Gelfree fraction into the Spin Filter. Add 200 µL Urea Sample Solution. Centrifuge at 14,000 x *g* for 25 min.
3. Add 200 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 12 min. Repeat this step once.
4. Discard the flow-through from the collection tube.
5. Add 10 µL 10X Iodoacetamide Solution and 90 µL Urea Sample Solution to the Spin Filter and vortex for 1 min; incubate without mixing for 30 min in the dark.
6. Centrifuge the Spin Filter at 14,000 x *g* for 12 min.
7. Add 100 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 10 min. Repeat this step twice.
8. Discard the flow-through from the collection tube.
9. Add 100 µL of 50 mM Ammonium Bicarbonate Solution provided with the FASP Kit to the Spin Filter and centrifuge at 14,000 x *g* for 10 min. Repeat this step twice.
10. Add 75 µL Digestion Solution (enzyme-to-protein ratio 1:100) and vortex for 1 min. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.
11. Incubate the Spin Filter in an incubator at 37 °C for 4 – 18 h.
12. Transfer the Spin Filter to a new collection tube.
13. Add 40 µL of 50 mM Ammonium Bicarbonate Solution to the Spin Filter and centrifuge at 14,000 x *g* for 10 min. Repeat this step once.
14. Add 50 µL 0.5 M Sodium Chloride Solution provided with the FASP Kit and centrifuge the Spin Filter at 14,000 x *g* for 10 min.
15. Filtrate contains digested protein fraction. Use a vacuum concentrator to dry the filtrate. Reconstitute sample in 20 µL of 0.1% formic acid.

REFERENCE

(i) Universal sample preparation method for proteome analysis. Wisniewski JR, Zougman A, Nagaraj N, Mann M. Nat Methods. 2009 May;6(5):359-62. Epub 2009 Apr 19.

DESCRIPTION	PART NUMBER
FASP Protein Digestion Kit.	44250

Contains materials for 8 digestions of up to .40 mg protein each
Eight spin columns, 16 collection tubes, 20 mL 100 mM Tris Hydro-chloride Solution pH 8.5, 8 single-use 0.75 g tubes Urea, 20 mL Ammonium Bicarbonate Solution, 1 mL 0.5 M Sodium Chloride Solution, eight single-use tubes Iodoacetamide.

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