expedeon
protein discovery & analysis

FFPE-FASP™ Protein Digestion Kit
USE AND STORAGE INSTRUCTIONS

INTRODUCTION

Expedeon's FFPE-FASP Protein Digestion Kit is for researchers who wish to analyze proteins in formalin-fixed paraffin-embedded (FFPE) tissue samples by mass spectrometry.

The FFPE-FASP Protein Digestion Kit provides the necessary columns and buffers to carry out the FASP-FFPE protocol similar to the one described by Ostasiewicz, Xielinska, Mann, and Wisniewski[i].

STORAGE AND STABILITY

Store FFPE-FASP Protein Digestion Kit materials at 4 °C. 2 year shelf life from the Day of manufacture.

USAGE GUIDELINES

• Start with 0.5 - 1.0 g FFPE tissue.

• For research use only.

RECOMMENDED PROCEDURE

MATERIALS NEEDED

FFPE-FASP Protein Digestion Kit
Snap-cap microfuge tube
Pipettor and Pipette Tips
Xylene solvent, 2 mL/tissue sample
Absolute ethanol, 2 mL/tissue sample
Trypsin or other proteolytic enzyme
Trifluoroacetic acid (TFA)
Benchtop centrifuge capable of 15,000 x *g*
Dounce homogenizer
Rocker or shaker for gentle agitation at room temperature
Thermal mixer or shaker for microfuge tube agitation at 105 °C
Incubator set at 37 °C
Centrifugal vacuum dryer

PREPARING UREA SAMPLE SOLUTION

Urea Sample Solution should be prepared fresh prior to digestion.

• Add 1 mL Tris Hydrochloride Solution provided with the FFPE-FASP Kit to one tube of Urea, also provided with the FFPE-FASP Kit. Vortex 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.

PROTOCOL

1. Place 0.5 g - 1.0 g FFPE tissue in the microfuge tube. Add 1 mL xylene solvent and incubate with gentle agitation at room temperature for 5 min.
2. Remove the solution, add 1 mL xylene solvent, and incubate as in (1).
3. Remove the solution and repeat steps (1) and (2) using absolute ethanol instead of xylene solvent.
4. Remove the solution and dry the sample using a centrifugal vacuum dryer.
5. Add 1 mL UPX Universal Protein Extraction Buffer provided with FFPE-FASP Kit per 50 mg dried tissue.
6. Homogenize the sample tissue with UPX Buffer in a dounce homogenizer for two to three minutes.
7. Incubate the homogenized sample with agitation at at 105 °C for 30 min.
8. Remove the tube from the heating block and allow it to cool slowly to room temperature.
9. Pellet the cellular debris by centrifuging the sample at 15,000 *g* for 10 minutes.
10. Mix up to 50 µL of the clarified lysate with with 200 µL Urea Sample Solution in the Spin Filter and centrifuge at 14,000 *g* for 30 min.
11. Add 200 µL Urea Sample Solution to the Spin Filter and centrifuge at 14,000 *g* for 20 min.
12. Discard the flow-through from the collection tube.
13. Add 90 µL Urea Sample Solution to the Spin Filter.
14. Add 10 µL 10X Iodoacetamide Solution to the Spin Filter and vortex for 1 min; incubate in the dark without mixing for 20 min.

15. Centrifuge the Spin Filter at 14,000 *g* for 10 min.
16. Add 100 µL Urea Sample Solution to the Spin Filter and centrifuge at 14,000 *g* for 15 min. Repeat this step twice.
17. Add 100 µL of 50 mM Ammonium Bicarbonate Solution provided with the FFPE-FASP Kit to the Spin Filter and centrifuge at 14,000 *g* for 10 min. Repeat this step twice.
18. Add 75 µL of 50 mM Ammonium Bicarbonate Solution with trypsin (enzyme to protein ratio 1:100) or another protease and vortex for 1 min. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.
19. Incubate the Spin Filter in an incubator at 37 °C for 4 - 18 h.
20. Transfer the Spin Filter to a new collection tube.
21. Centrifuge the Spin Filter at 14,000 *g* for 10 min.
22. Add 50 µL of 50 mM Ammonium Bicarbonate Solution and centrifuge the Spin Filter at 14,000 *g* for 10 min.
23. Add 50 µL of 0.5 M Sodium Chloride Solution provided with the FFPE-FASP Kit and centrifuge at 14,000 *g* for 10 min.
24. Filtrate contains digested protein fraction. Acidify with TFA to desired pH and desalt.

REFERENCE

(i) Proteome, phosphoproteome, and N-glycoproteome are quantitatively preserved in formalin-fixed paraffin-embedded tissue and analyzable by high-resolution mass spectrometry. Ostasiewicz P, Zielinska DF, Mann M, Wiśniewski JR. J Proteome Res. 2010 Jul 2; 9(7): 3688-700.

ORDERING INFORMATION

To order the FFPE-FASP Protein Digestion Kit, visit www.expedeon.com to purchase online, or contact

DESCRIPTION	PART NUMBER
FASP-FFPE Protein Digestion Kit. Contains materials for eight digestions Eight spin columns, 16 collection tubes, 10 mL UPX™ Universal Protein Extraction Buffer, 100 mM Tris Hydrochloride Solution pH 8.5, eight single-use 0.75 g tubes Urea, 20 mL Ammonium Bicarbonate Solution, eight single-use tubes Iodoacetamide, 1 mL 0.5 M Sodium Chloride Solution.	44255

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