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ab270543 FASP Protein Digestion Kit (FFPE)

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 44255

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FASP Protein Digestion Kit (FFPE) datasheet:
www.abcam.com/ab270543
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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 (FFPE) (ab270543) allows unbiased extraction, complete proteome solubilization, and highly efficient digestion of FFPE tissue. The resulting filtrate is free of detergents, large molecules, and other substances that would interfere with MS analysis of the proteome.

Filter Aided Sample Prep (FASP) is the enabling technology behind quantitative mass spectrometry (MS) analysis of archived tissues. Based on a spin filter sample preparation method initially described by Manza, et al., and developed further and extended to FFPE tissue processing by Ostasiewicz, et al.

2. Materials Supplied and Storage

Store at +4°C immediately on receipt. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	8 pack	Storage temperature (before prep)
Tris-HCl Solution (50 mM)	1 bottle (20 mL)	+4°C
Urea	8 x 0.75 g vials	+4°C
Iodoacetamide	8 x 9.8 mg vials	+4°C
Protein Extraction Buffer	1 bottle (50 mL)	+4°C
Ammonium Bicarbonate Solution (50 mM)	1 bottle (20 mL)	+4°C
Spin Filter (30 kDa MWCO)	8	+4°C
Sodium Chloride Solution (500 mM)	1 bottle (1 mL)	+4°C

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
- 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 *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
- Collection tube

4. Reagent Preparation

Prepare fresh reagents immediately prior to use.

4.1 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.

4.2 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.

5. Assay Procedure

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

Proteome Extract Digestion protocol:

5.1 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 minutes.

5.2 Remove the solution, add 1 mL xylene solvent, and incubate as in step 7.1.

5.3 Remove the solution and repeat steps 7.1 and 7.2 using absolute ethanol instead of xylene solvent.

5.4 Remove the solution and dry the sample using a centrifugal vacuum dryer.

5.5 Add 1 mL Protein Extraction Buffer provided per 50 mg dried tissue.

5.6 Homogenize the sample tissue with Protein Extraction Buffer in a dounce homogenizer for two to three minutes.

5.7 Incubate the homogenized sample with agitation at 105°C for 30 minutes.

5.8 Remove the tube from the heating block and allow it to cool slowly to room temperature.

5.9 Pellet the cellular debris by centrifuging the sample at 15,000 x *g* for 10 minutes.

5.10 Mix up to 50 µL of the clarified lysate with 200 µL Urea Sample Solution in the Spin Filter and centrifuge at 14,000 x *g* for 30 minutes.

5.11 Add 200 µL Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x *g* for 20 minutes.

5.12 Discard the flow-through from the collection tube.

5.13 Add 90 µL Urea Sample Solution to the Spin Filter.

5.14 Add 10µL 10X Iodoacetamide Solution to the Spin Filter and vortex for 1 minute; incubate in the dark without mixing for 20 minutes.

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

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

5.17 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.

5.18 Add 75 µL of 50mM Ammonium Bicarbonate Solution with trypsin (enzyme to protein ratio 1:100) or another protease and vortex for 1 minute. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.

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

5.20 Transfer the Spin Filter to a new collection tube.

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

5.22 Add 50 µL of 50 mM Ammonium Bicarbonate Solution and centrifuge the Spin Filter at 14,000 x *g* for 10 minutes.

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

5.24 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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