BIOGNOSYS
PROTEOMICS – NEXT GENERATION

Sample Preparation Kit

Extension protocol for frozen tissues

The Sample Preparation Kit is a simple-to-use standardized kit for reproducible mass spectrometry proteomics of cell cultures and plasma/serum samples. The protocols were tested for different mass spectrometry applications such as SRM/MRM, HRM/DIA/SWATH and shotgun MS/MS and showed good performance.

Here we describe the extension of the sample preparation procedure that enables the processing of frozen tissue samples. The kit components are sufficient for 20 frozen tissue sample preparations to be made at once. Buffers are unstable and thus not appropriate for long-term storage once they are dissolved.

Sample preparation procedure for frozen tissues should be started with this extension.

Sample requirements

Frozen tissue sample with dimensions 0.2x0.4cm, and thickness 0.2mm.

A. Denaturation, reduction and alkylation

1. Dissolve 10x Dilution Buffer with water to a total volume of 5 ml, vortex until solubilized.
2. Prepare Denature Buffer:
   2.1. Add 500 µl of 10x Dilution Buffer to the Denature Buffer tube. Keep 10x Dilution Buffer in fridge until further usage.
   2.2. Fill up Denature Buffer with 2.3 ml of water.
3. Add 200 µl of Denature Buffer and 25 µl of RapiGest detergent (Waters) (1% RapiGest in 250mM ammonium bicarbonate) to each frozen tissue sample.
4. Grind in bead mill (e.g. Tissuelyser II from Qiagen), one stainless bead per 2ml tube used, homogenization at a frequency 30/s for 3x1 min.
5. Spin down at 10,000g to remove remaining solid material, use supernatant for further processing.
6. Leave the samples on room temperature for 15 min.
7. During incubation you can measure the total protein concentration using a total protein assay (like BCA or Bradford) according to the manufacturer's protocol.
8. Prepare 1x Dilution Buffer by mixing in a separate 15 ml tube 1 ml of 10x Dilution Buffer and 9 ml of water.
9. Dilute the samples with 1x Dilution Buffer to obtain 100 µl per sample with a protein concentration of 1 µg/µl. Keep 1x Dilution Buffer in fridge until further usage.

Optional: If convenient you can freeze your samples on -20ºC. After thawing vortex the samples for 2 min before further use.

At this point continue with point 8 (B. Reduction and alkylation) of the Cell Culture Sample Preparation Procedure on page 9 of the Sample Preparation Kit Manual available at:

http://www.biognosys.ch/shop/sample-preparation-kit

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