denator
STABILIZING THE PROTEOME

Stabilizor®

Peptide extraction kit, Tissue

User Manual: LC-MS

• Introduction

Analysis of endogenous peptides in biological tissues is easily compromised by the rapid emergence of *ex vivo* protein degradation fragments. Stabilization of tissue samples in the Stabilizor™ system inactivates the enzymes that cause sample degradation and thereby prevent Protein/peptide changes.

This protocol focuses primarily on extraction of water soluble neuropeptides from brain tissue – molecules particularly prone to rapid degradation. However, the protocol is also valid for other sample types.

• Materials supplied

- 12 x Maintainor™ Tissue cards
- 12 x 10kDa Cut-off spin filters
- 24 x Tubes for homogenization/centrifugation
- 3 x 45 ml Extraction fluid
- User Manual: LC-MS

• Storage

- Store kit at room temperature
- Use Extraction Fluid within 24 months from date of manufacture (see label)
- After opening, store Extraction Fluid at +4°C-+8°C and use within one month

• Required equipment (not provided)

- Stabilizor T1 instrument
- Adjustable pipette (1000µl)
- Analytical balance scale
- Homogenization device
- Centrifuge (14,000 x g)
- 1.5 ml centrifuge tubes

• Safety information

Concentrations of hazardous compounds in the Extraction Fluid are below those set by REACH Regulation (1907/2006) as requiring Safety Data Sheets. A Safety Data Sheet can be supplied upon request.

• Filter limitations

Filters are non-autoclavable.
Do not centrifuge above 14,000 x g.
Filter membranes contain trace amounts of glycerin. If glycerin will interfere with downstream analysis, pre-wash filters by centrifugation of the assembled filter device using dH_2O or extraction buffer. After washing, do not let filter membrane dry out completely before use.

• Sample preparation

Avoid unnecessary freeze-thaw cycles and variations in sample handling. Stabilized samples are not sensitive to enzymatic activity; however non-enzymatic processes such as oxidation can still affect the samples.

• Technical assistance

For questions about this kit as well as the use of the Stabilizor instrument and working with heat-stabilized tissue, visit www.denator.com for up to date details of application and technical support in your region.

Products are for research use only

Protocol

Sample collection

1. Prepare all equipment and solutions prior to sampling.
2. Extract sample and place centrally in a Maintainor Tissue card.
3. Stabilize immediately in the Stabilizor system using the selected program mode.
   ! *Samples can be frozen and stabilized at a later stage if preferred*
4. Store stabilized samples in a freezer if they are not to be analyzed directly.
   ! *Stabilized samples can be dissected if required. Avoid pieces smaller than 20 mg as some loss may occur in the spin filter.*
5. Transfer samples to pre-weighed spin filter collection tubes.
   ! *Keep frozen samples on dry ice while weighing collected tubes.*
6. Weigh samples and calculate sample weight.

Homogenization

Samples must be thoroughly homogenized to facilitate peptide solubilisation.
Microtip sonication works well when homogenizing stabilized brain tissue.

1. Add extraction buffer, 5µl/mg of sample.
2. Homogenize e.g microtip sonication 3x4 seconds in an ice bath.
3. Centrifuge extract at top speed for approximately 30 minutes to clarify suspension and pellet cell debris.
4. Collect supernatant.

Protein concentration measurement

For semi-quantitative measurements

Use a standard kit such as the BCA™ Protein Assay Kit (Pierce) to part of each sample. Keep other part frozen during analysis. Add extraction buffer to level out any concentration differences.

Peptide separation

1. Assemble filter and centrifuge tube – insert white end of filter into the centrifuge tube.
2. If necessary, pre-wash filter by adding 50µl extraction buffer and centrifuge at 14,000 x g, for 15 minutes. Remove filter and put it into a new collection tube.
   ! *When using pre-washed tubes, use equal volumes of extract from all samples in step 3.*
3. Carefully decant 100 - 500µl, of extract and pipette into the filter reservoir.
   ! *Do not touch filter with the pipette tip.*
   ! *Ensure that particulate matter does not enter the reservoir as this may clog the membrane.*
4. Centrifuge at 14,000 x g for 60 minutes.
5. If extracting from small samples (<20 mg), wash filter once by adding 25µl of extraction buffer and centrifuge again at 14,000 x g for 15 minutes.
6. Remove filter from the centrifuge tube.
7. Store filtrate containing peptide extract for subsequent use.

LC-MS and analysis

Subsequent downstream analysis is not affected by using the Stabilizor system. Conventional LC-MS protocols can be followed without alteration.

Reference:
Heat Stabilization of the Tissue Proteome: A New Technology for Improved Proteomics
M. Svensson et al. J. Proteome Res., 2009, 8 (2), pp 974–981

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User Manual, Denator 60007 REV 4
To be used together with DKT0001.