Technical Note

Extraction of proteins from heat stabilized tissue samples

Introduction

This technical note provides general guidelines on how to achieve the most efficient extraction of proteins from heat-stabilized tissue. Specific protocols for heat stabilization, homogenization and extraction prior to LCMS or 2D-GE analysis are supplied with the Stabilizor™ Peptide Extraction Kit and Stabilizor 2D-GE Extraction Kit respectively.

Heat stabilization of tissue samples instantly and permanently inactivates enzymatic activity thereby preventing further degradation and changes downstream. Subsequent extraction methods must take into account that rapid, homogeneous heating to a high temperature completely denatures proteins in the sample. When extracting proteins from heat stabilized samples the following points must be observed:

Use a denaturing buffer (>8M Urea, >1% SDS, >6M GuHCl, SDS)

Buffers based on >8M urea, >6M GuHCl or >1% SDS have proven to be effective.
When using SDS buffers, it is recommended to maximize their effectiveness by adding heated buffer (>90°C) to the sample, preferably followed by a second homogenization step using ultrasonication. Other components such as detergents and buffering agents can be added as long as the concentrations of the denaturing agents are not affected.

Use a buffer-to-sample ratio greater than 10 (>10µl buffer/mg sample)

For maximum extraction efficiency it is important to add at least 10 times the amount of sample, *i.e.* 10 µl of buffer for each mg of sample. Use pre-weighed sample containers to minimize handling.

Ensure thorough homogenization using ultrasonication, grinding or ball mill

Although heat stabilized samples will usually homogenize easily, it is very important to ensure that the initial homogenization step is thorough to facilitate the resolubilization of proteins. Soft tissue such as brain can be homogenized using a microtip sonication rod, whereas a micropestle grinding or ball mill should be used for firmer tissues such as liver or heart. A combination of physical homogenization followed by rod sonication is recommended for best extraction.

Downstream analysis

Other parameters may need to be considered depending on subsequent downstream application:

- Ensure buffer compatibility with the protein quantification technique
- Note that high concentrations of urea, GuHCl and SDS may interfere with some analytical procedures
- Use dilution to reduce urea concentration rather than clean-up cartridges or dialysis which may cause protein precipitation

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