Recommended protocol

SDS-PAGE & Western blot

Introduction

Protein analysis in biological matrices is sometimes compromised by sample degradation. When using the Stabilizor™ system, active enzymes causing protein degradation is halted and thereby the protein content is unaltered. However, when performing gel electrophoresis on stabilized samples it is important to homogenize each sample thoroughly. Consideration must also be taken to the selection of extraction buffer and the buffer-to-sample ratio. The best results are found when extraction is performed in denaturing buffers and the buffer-to-sample ratio is greater than 10, *i.e.* at least 10 µl of buffer for each mg of sample.

The following protocol works very well together with stabilized samples; however, other protocols may also be applicable but should be tested for compatibility with the Stabilizor system and downstream assays. The protocol is primarily written with tissue samples in mind but a majority of the steps is also valid for other sample types.

Specific protocol

Sample collection

1. Make sure that everything is ready for stabilization prior to sampling.
2. Extract sample and place in Maintainor® Tissue card.
2. Immediately stabilize in Stabilizor instrument according to Stabilizor manual using the appropriate program mode.
   Alternatively, the sample can be frozen and stabilized at a later stage.
3. If necessary, sub-dissect samples after stabilization but before freezing.
4. If you do not intend to analyze the samples directly, store samples at -80°C.
5. Weigh samples together with pre-weighed centrifugation tubes. If you intend to use pestles and grinding for sample homogenization, avoid collecting samples larger than 50 mg per 1.5 ml tube, because of the risk of sample loss during open-tube homogenization.
6. Calculate sample weight.

Extraction buffer

For SDS-PAGE, stabilized samples should be extracted in hot 1% SDS buffer. Add the extraction buffer hot (>90°C) to maximize solubilization of heat denatured proteins. Add at least 10 µl of extraction buffer for each mg of sample. For samples less than 10 mg use at least 100 µl of buffer.

Homogenization

Stabilized samples generally homogenize well but it is very important that the samples are thoroughly homogenized to facilitate solubilization of proteins. Soft tissue such as brain can be homogenized using a microtip sonication rod whereas firmer tissues such as liver or heart need a more rigid homogenization method such as grinding or ball mill pulverization before any sonication. The combination of physical homogenization followed by rod sonication is recommended for best extraction.

Sample grinding using pestle for 1.5 ml tube

1. Use a pestle directly in an ordinary 1.5 ml centrifugation tube without the abrasive material (if any). It is commonly included abrasive material to sample grinding kits; however, this does not increase extraction efficiency for stabilized tissue samples.
2. Crush sample coarsely prior to adding extraction buffer.
3. Add 10 µl of extraction buffer for each mg of sample. Add the buffer in two steps and homogenize after each addition. Start by adding 30% of intended buffer and after initial homogenization, add the remaining buffer and continue homogenization.
4. Homogenize for at least 2 min using pestle; continue even when no traces of coarse material remain.
5. An additional sonication step is recommended to ensure complete solubilization of proteins.
6. Centrifuge at top speed for 25 min to sediment cellular debris.
7. Carefully recover supernatant into a new tube without disturbing the pellet.

Ball mill

1. Homogenization is preferentially done frozen, with tissue pieces smaller than 1 mm^3. The tube holder and the sample tube, together with balls, should be cooled in liquid nitrogen prior to adding the sample.
2. Homogenize frozen sample for 1 min at maximum frequency.
3. Add 10 µl of extraction buffer for each mg homogenized sample, while balls are still in tube. Mix while sample thaws and remove balls using a magnet when they are no longer covered with sample.
4. An additional sonication step is recommended to ensure complete solubilization of proteins.
5. Centrifuge at top speed for 25 min to sediment cellular debris.
6. Carefully recover supernatant into a new tube without disturbing the pellet.

Sonication

1. Using a microtip, sonicate at 40 W output for at least 20 s or until no traces of coarse material remain, preferably in pulses of 2 s.
2. Centrifuge at top speed for 25 min to sediment cellular debris.
3. Carefully recover supernatant into a new tube without disturbing the pellet.