Application Note

Seamless compatibility between Stabilizor system and Western SDS-PAGE

Introduction

After sampling, proteases and other protein-modifying enzymes can rapidly change proteome composition. Vital information about the 'pre-sampling' state may be destroyed or distorted. The Stabilizor™ system uses a combination of heat and pressure to completely inactivate enzymes in tissue samples without the need of any additives. Compatibility with downstream analytical methods is crucial, regardless of sample preparation technique. Here, the compatibility between Stabilizor and two commonly used downstream analysis techniques for protein analysis, SDS-PAGE and Western Blot is investigated and shown to be seamless.

Methods

Small pieces of mouse brain cortex, liver and heart were rapidly heat inactivated using the Stabilizor instrument or snap frozen. Using a ball mill (Retsch) or Tissue Grind Kit (GE Healtcare) samples were homogenized and extracted into 5 x sample weight of 1% SDS extraction buffer. Samples were heated for 10 min at 95°C, interrupted by sonication every other minute, 5x 2 sec bursts at 40 W energy setting (Vibra-Cell, Sonics & Materials). Protein content was measured using 2D Quant Kit (GE Healthcare) following standard instructions. Proteins were visualized using GelCode™ Blue Stain Reagent (Pierce).

Western Blot

Protein samples of 20 µg were loaded onto 4-20% TGI acrylamide gels (Invitrogen), separated by SDS-PAGE and transferred to 0.2 µm nitrocellulose membranes (Immobilon-PSQ, Millipore). The membranes were immunoblotted using antibodies recognizing total MAPK, MEK and AKT (Abcam).
Antibody binding was shown by incubation with goat antirabbit horseradish peroxidase-linked IgG (Pierce) and ECL immunoblotting detection system (GE Healthcare).
Chemiluminescence was detected by using DuPont NEN autoradiography film (Sigma) and levels quantified by densitometry (National Institutes of Health IMAGE 1.61 software). Analysis was repeated with five independent replicas.

Results and conclusion

Heat stabilization using the Stabilizor instrument has no significant effect on global protein content, as visualized by SDS-PAGE, compared to snap frozen samples. This is further shown by equal protein levels for the three proteins specifically selected for western blot analysis.

Figure 1A demonstrates clearly that, by following identical extraction procedures, equal levels of protein can be extracted from snap frozen and stabilized tissue samples. In addition, no significant difference in protein profiles (Figure 1B) is seen between snap frozen and stabilized tissue.

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Jan 2016

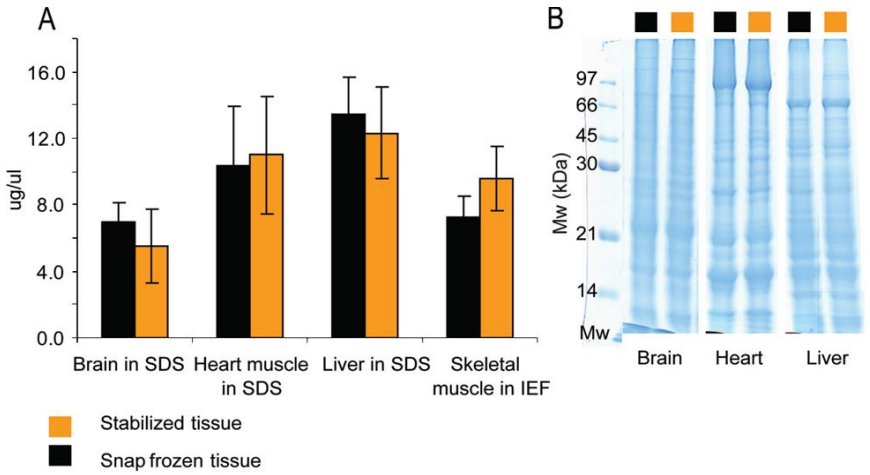

Fig 1. (A) Levels of extracted protein from mouse brain, heart, and liver in hot 1% SDS or heart in standard proteomics extraction buffer. Stabilized samples were treated for 45 s at 95 °C with the Stabilizor T1 system (n =5). (B) GelCode Blue stained SDS-PAGE. The proteins in mouse cortex, heart, and liver were extracted in 1% hot SDS and separated together with a size marker. Lanes indicated with orange denote stabilized samples

Western blot analysis (Figure 2) with antibodies against MAPK, MEK, and AKT show very similar levels of the three proteins extracted from snap-frozen as well as stabilized tissue for all three tissues tested.

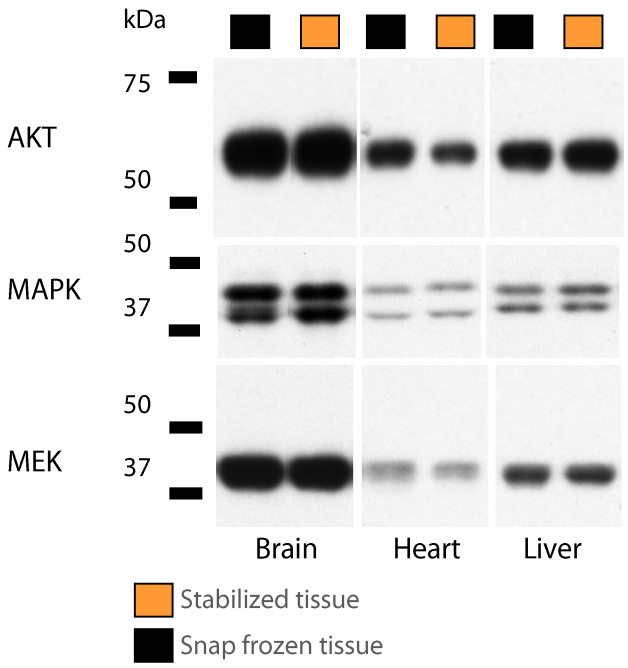

Fig 2. Panels showing Western Blot images of levels of AKT, MAPK, and MEK in mouse brain cortex, heart and liver. Lanes marked by orange squares indicate that samples were stabilized immediately post-dissection, Lanes marked by black squares indicate that samples were snap frozen in liquid nitrogen immediately post-dissection. Representative Western blot images are inserted over corresponding columns. Analysis was repeated five times with independent biological replicates with similar outcome