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Recommended protocol

Stabilization of liquid samples using Maintainor Liquid

Introduction

When stabilizing liquid samples, *e.g*. CSF, plasma and cell suspensions, in the Stabilizor™ system, active enzymes, causing protein degradation, are inactivated by heat and the protein/peptide/metabolite content remains unaltered. Due to high protein content in blood based samples, they will coagulate during stabilization. The coagulated, solidified, sample can be resolubilized in a denaturizing buffer. Depending on downstream application the processed samples may have to be diluted in order to be compatible with the intended downstream process due to high urea content in the resolubilization buffer. The advice below are made to the best of our current knowledge. However, stabilization of liquid samples and subsequent analysis is an experimental procedure that may require optimization to work well.

Specific protocol

Treating liquid samples in the Maintainor® Liquid card

1. Run sample in Custom mode in Stabilizor instrument according to following parameters:
    a. Set Heater temp to 95°C
    b. Set Upper heater distance to 2.1 mm (or lowest possible value)
    c. Set Cavity vacuum OFF
    d. Set treatment time to 45 seconds.
2. Position a Maintainor Liquid card on a flat surface.
3. Place up to 1 ml of sample in the card and close it, ensuring it is sealed all around.
4. Place card into the sled and press START.
5. After stabilization, blood based samples will be coagulated. Open the card carefully and use a spatula to transfer the coagulated sample to an Eppendorf tube for further processing.
6. For non-coagulating liquids, either use a sharp object to open the cavity by piercing the upper foil or alternatively, peel away part of the back foil to get access to the cavity and use a pipette to recover the sample. Pierce the foil close to the rim of the cavity away from the liquid and use a pipette to recover the sample. Opening the card the standard way may cause the content to spill.

Resolubilization of stabilized blood based samples

1. Stabilized samples can be resolubilized using urea based buffer in combination with rod sonication. We recommend the following denaturizing buffer; 8 M urea, 20mM TRIS, pH8. (As long as the buffer contains at least 8M urea composition of other components can vary.)
2. Add three to five (3-5) volumes of extraction buffer for each volume of stabilized plasma.
3. Preferentially use a rod sonicator to dissolve the stabilized plasma. Eppendorf tube micro-pestle homogenizer followed by vortexing will also work but resolubilization is much slower.
4. Centrifuge the tube at maximum speed, e.g. 13 000xg, for 20 minutes to remove insoluble material.

5. Collect supernatant and proceed with analysis. Due to high level of urea, samples may need to be diluted depending on downstream analysis technique.