NDMRB-SOP-036

Processing of tissue samples within the Mass Spectrometry Research group

1.0 Introduction

This document outlines the general procedure employed to render samples acellular within 7 days of receipt into the Mass Spectrometry research group of Prof. Benedikt Kessler in the Target Discovery Institute.

This procedure is linked, in particular, to samples brought or sent to the NDMRB by collaborators working with Prof. Benedikt Kessler. All other samples fall outside of the scope of this SOP.

2.0 Overview of the Procedure

Upon receipt, tissue samples must be logged into tissue sample database, at the following location N:\Proteomics\Tissue Sample Database\SRF. Samples should be stored in the designated location as stated in the database at -80°C.or -20°C
Within 7 days of receipting the tissue samples, the tissue will be treated by one of the following means to render samples acellular.

3.0 Procedure for Rendering Cells Acellular

One of the following methods should be employed to ensure samples are made acellular. This procedure outlines the general steps.

3.1 Fluid samples

- Centrifuge fluid samples (e.g. urine, blood, plasma) for sufficient time and speed to pellet the cellular component. The centrifuge speed and time will vary according to the tissue sample type, as a guide, centrifuge samples for minimum 10 minutes at 3000RPM at 4°C. Repeat the centrifuge process, if samples remain turbid.
- Remove the supernatant after centrifugation has been complete.
- Continue to standard Proteomics/Metabolomics sample preparation protocols using the supernatant

- If the cell pellet is not required for sample processing, discard the tube containing the pellet to the clinical waste bins. Solid waste will autoclaved at 136°C for 12 minutes and then incinerated.
- If the cell pellet is required for analysis, proceed to **Section 3.2 Solid Tissue Samples**

3.2 Solid Tissue Samples

Solid tissue samples should be processed to remove the cellular component.
The most common method is through a combination of mechanical and chemical lysis.

- Place tissue samples on ice prior to addition of a suitable lysis buffer. The ratio of lysis buffer to tissue should be taken in to consideration for optimal lysis. For the most common lysis buffer, RIPA (**R**adio**I**mmuno**P**recipitation **A**ssay Buffer) add at a ratio of 0.4ml per 0.1g tissue.
- Homogenize on ice using one or a combination of the following a tight-fitting glass homogenizer, bead beater, sonicator, and/or extensive vortexing.
- Incubate on ice for minimum 10 minutes to maximize protein solubilization.
- Centrifuge at 17,000g at 4°C for 10 minutes, extend this time if a pellet has not formed or the sample remains turbid.
- Transfer the supernatant to clean Lobind tubes, and proceed to In Solution Digestion protocol.
- Discard the tubes containing the pellet to the clinical waste bins or Dispo containers. Waste will be autoclaved at 136°C for 12 minutes, followed by third party waste collection for incineration..

4.0 Safety Consideration

Treat samples as potentially infectious material and handle under a Biological Safety Cabinet.
Good Laboratory Practices apply as per local rules NDMRB-POL-008.
Use of Laboratory Centrifuges TDI-SOP-009
Use of Sonicators TDI-SOP-009 & TDI-RA-003
Working with Blood, Urine and Human Tissue TDI-RA-043
Handling of Biological Material TDI-SOP-007

5.0 Storage and Disposal

Track all human tissue samples, if they are made acellular, disposed or retain as whole tissue in the database Samples are tracked at the following location;
N:\Proteomics\Tissue Sample Database\SRF Samples are stored at -80°C

Samples are disposed of as per local rules NDMRB-POL-006:

- Liquid waste is treated with 1% Virkon solution for a minimum of 15 minutes before being disposed down the drain with copious amounts of water.
- Solid waste is autoclaved at 136°C for 12 minutes before being disposed of via third party waste collection for incineration.

6.0 Reference

6.1 Human Tissue Authority : relevant material

https://www.hta.gov.uk/policies/relevant-material-under-human-tissue-act-2004

7.0 Review

The information in this document will be reviewed and amended if necessary every 3 years by the laboratory manager or alternative relevant personnel.