Dublin Academic Medical Centre

Standard Operating Procedure
Dublin Academic Medical Centre
UCD Clinical Research Centre

SOP Number	**5.7**
Version Number	**1**
SOP Title	**Processing of Tissue**

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Purpose

To outline the laboratory procedures for extracting DNA, RNA and Protein from tissue.

Specific procedure

1. Tissue samples can be stored in liquid nitrogen or at -80oC, as paraffin-embedded tissue or as formalin fixed tissue.

DNA extraction from Tissue

DNA extraction from tissue samples stored frozen shall be similar to the SOP 5.1.

DNA extraction from paraffin embedded tissue should follow the subsequent procedure:

a) Lysis time will vary from sample to sample depending on the type of tissue processed
b) Yields will depend both on the size and the age of the sample processed. Reduced yields compared fresh or frozen tissue, are expected. Therefore eluting the DNA in 50-100µl Buffer is recommended.
c) Place a small section (not more than 25mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tube
d) Add 1200 µl xylene or toluene. Vortex vigorously.
e) Centrifuge at full speed for 5 min at room temperature.

f) Remove supernatant by pipetting. Do not remove any of the pellets.

g) Add 1200 ml ethanol (96-100%) to the pellet to remove residual xylene or toluene and mix gently by vortexing.

h) Centrifuge at full speed for 5 min at room temperature.

i) Carefully remove the ethanol by pipetting. Do not remove any of the pellets.

j) Repeat the previous steps.

k) Incubate the open microcentrifuge tube at 37oC for 10-15 min until the ethanol has evaporated

l) Resuspend the tissue pellet in 180 µl of buffer and follow the procedure described in SOP 5.1.

DNA extraction from formalin fixed tissue should follow the subsequent procedure:

a) Wash tissue sample twice with PBS to remove fixative.

b) Process sample according SOP 5.1

RNA extraction from Tissue

RNA extraction from tissue shall follow the procedure described in SOP 5.2. Before the extraction procedure, the tissue should be collected in RNAlater solution and then homogenized in 1ml TRI reagent per 50-100mg

tissue. Also, the RNA can also be extracted from formalin fixed and wax embedded tissue.

Protein extraction from Tissue

Protein extraction shall be performed from frozen tissue following the procedures described in SOP 5.3.

Collected tissue for primary cell culture shall be processed in the same day. Procedures are different depending the type of tissue and the type of cells to be cultured. Refer to the specific protocol for the procedure applied.

Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.