LOWY BIOREPOSITORY STANDARD OPERATING PROCEDURE FOR TISSUE DERIVATIVES: DNA EXTRACTION

INTRODUCTION & SCOPE

The purpose of this document is to outline the standardised procedure undertaken by the Lowy Biorepository for the extraction of DNA from tissue samples. This Standard Operation Procedure (SOP) describes how molecular derivatives are extracted to ensure the process is completed in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity.

The tissue samples collected from patients who have given their informed consent are stored at the Lowy Biorepository until requested for Human Research Ethics Committee (HREC) approved research. On request, molecular derivatives are extracted from the tissue specimens with an aliquot provided for research.

This SOP does not cover detailed safety procedures for handling human biological materials or hazardous chemicals.

SAFETY CONSIDERATIONS

The Lowy Biorepository is a PC2 laboratory and correct PPE must be worn when handling all biological specimens. All tissue is treated as potentially infectious.

PROCEDURAL STEPS

1. Labelling
    a. Specimens are logged into the caTissue database and assigned specimen numbers and box/spot positions within the –80°C freezer prior to processing/storage.
    b. Post processing, DNA vial labels are generated from the caTissue database and printed directly onto Brady labels.
    c. Minimum data on labels:
        i. Specimen number
        ii. caTissue Collection Protocol title
        iii. Specimen type – DNA
        iv. Concentration – ng/uL
        v. Lowy Biorepository box number & spot location
2. Processing
    a. Transfer cut section of tissue into 450 uL TESS lysing agent and use a micro pestle to disrupt the tissue.
    b. Add 0.5 mg/ml proteinase K and vortex for 2 sec.
    c. Incubate at 55°C overnight (minimum 16 hr) in a heat block OR for urgent same-day extractions perform the incubation at 60°C for 4 hr.

d. Centrifuge (13000 rpm/5 min/RT).

e. Immediately prior to use, pellet Phase Lock Gel (PLG) tube (13000 rpm/5 min).

f. Add aqueous sample (500–600 uL) to PLG tube.

g. Transfer PLG tube to fume hood. Add 450 uL phenol:chloroform premixed w/isoamyl alcohol (25:24:1). Do not vortex.

h. Centrifuge (13000 rpm/5 min).

i. Add 25 ug/mL (final concentration) RNase A to each PLG tube into the aqueous phase. Mix gently and incubate for 45–90 min at 37°C.

j. Add aqueous sample (500–600 uL) to PLG tube.

k. Transfer PLG tube to fume hood. Add 450 uL phenol:chloroform premixed w/isoamyl alcohol (25:24:1). Do not vortex.

l. Centrifuge (13000 rpm/5 min).

m. Add 450 uL chloroform to the aqueous sample. Do not vortex.

n. Centrifuge (13000 rpm/ 5 min).

o. Transfer the upper aqueous phase to a clean 1.5 ml micro tube (labeled with DNA number). Add 2 volumes ice-cold absolute ethanol and 1/10 volume 3M sodium acetate. Invert tube gently until a DNA precipitate is observed.

p. If no precipitate is observed, add 1 ug/ul glycogen and place 1.5 ml vial at –80°C for 60 min or at –20°C overnight.

q. Centrifuge (13000 rpm/ 30 min).

r. Carefully remove supernatant, do not disturb DNA pellet.

s. Add 1 mL of 70% ethanol.

t. Centrifuge (13000 rpm/ 5min).

u. Remove supernatant and allow the micro tube to air dry for 30 min.

v. Resuspend DNA pellet in an appropriate volume of 1 X TE (depending on initial tissue size and the size of the visible DNA pellet).

3. Quantitation/aliquoting

a. Quantitate DNA using the NanoDrop® ND-1000 Spectrophotometer.

b. Enter data into caTissue to generate labels.

c. Aliquot requested amount for research and distribute as per protocol.

d. Box/spot locations on the DNA vials are cross checked prior to placement in the –80°C freezer.

EQUIPMENT MONITORING

All banked DNA specimens are stored in –80°C freezers that are monitored and connected to the Lowy Cancer Research Centre alarm system. An additional –80°C back up freezer is running continuously.

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