LOWY BIOREPOSITORY STANDARD OPERATING PROCEDURE FOR BLOOD DERIVATIVES: PLASMA & DNA EXTRACTION

INTRODUCTION & SCOPE

Peripheral blood samples are collected from patients who have provided informed consent. These samples are stored as cell pellets until requested for Human Research Ethics Committee approved research. On request, molecular derivatives are extracted from these blood specimens and an aliquot provided for research.

The purpose of this document is to outline the standard operating procedure (SOP) undertaken by the Lowy Biorepository when extracting DNA from whole blood/cell pellet samples to ensure that DNA is extracted in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. The 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 blood 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. DNA should be extracted from blood within 1–5 days of collection.
   b. Perform all Day 1 manipulations in a Class II biological safety cabinet.
   c. Centrifuge blood tube (2000 rpm/10 min) and remove the plasma (and aliquot to 1mL and store at –80°C if required) to within 1 cm of the buffy coat with a transfer pipette.

d. Collect the buffy coat into a 15 mL centrifuge tube with a transfer pipette. Transfer the buffy coat layer and 1 cm above (plasma) and 1 cm below (erythrocytes) the buffy coat layer. 2–3 mL of buffy coat per 9 mL blood tube is collected.

e. Add 1 x OTL to 14 mL. Mix by gentle inversion. Incubate at room temperature for 10 min. Centrifuge (2000 rpm/5 min/RT). Repeat this step.

f. Add DPBS to 14 mL mix by gentle inversion

g. Centrifuge (2000 rpm/5 min/RT).

h. Add 2 mL TESS.

i. Add 0.5 mg/mL (final concentration) proteinase K. Mix by gentle inversion.

j. Incubate 15 mL centrifuge tube at 55°C overnight (minimum 16 hr) in a heated water bath. For urgent same-day extractions perform the incubation at 60°C for 4 hr.

k. Centrifuge (4000 rpm/5 min) to pellet cellular debris.

l. Pellet Phase Lock Gel (PLG) tube contents at 4000 rpm for 5 min.

m. Add lysed PBMC (approx 2 mL) to PLG tube.

n. Transfer PLG tube to fume hood. Add 2 mL phenol-chloroform premixed w/isoamyl alcohol (25:24:1) and thoroughly mix the organic and aqueous phases to from a transiently homogenous suspension. Do not vortex.

o. Transfer PLG tubes to centrifuge buckets in fume hood. Fit aerosol tight caps to buckets.

p. Centrifuge (4000 rpm/5 min).

q. Transfer PLG tube to fume hood (in buckets fitted with aerosol tight caps). Add 250U (final concentration) RNase. Mix gently by inversion. Incubate for 45–90 min at 37°C, in a heat block or water-bath in the fume hood

r. Centrifuge (4000 rpm/5 min)

s. Add 2 mL chloroform to the aqueous sample and thoroughly mix. Do not vortex.

t. Centrifuge (4000 rpm/5 min).

u. Add 2 volumes ice-cold absolute ethanol and 1/10 volume 3M sodium acetate Invert tube gently until a DNA precipitate is observed. (If no precipitate is observed add 1 ug/ul glycogen and place at –80°C for 60 min or at –20°C overnight).

v. Transfer the precipitated DNA, with a P200 tip, to 1 mL of 70% ethanol and centrifuge (13000 rpm/30 min).

w. Carefully remove supernatant with a P1000 pipette tip. Do disturb DNA pellet.

x. Allow the DNA pellet to air dry for 30–60 min.

y. Resuspend DNA pellet in an appropriate volume of 1 X TE (depending on 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.

Author: Biorepository Manager

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