Imperial College London

MULTI-'OMICS ATLAS PROJECT

March 2023

Standard operating procedure: RNA extraction from fresh frozen human brain tissue using QIAzol.

Health and Safety hazards and control measures

- Hazardous Material – Unscreened, unfixed human tissue.

User must wear gloves and laboratory coat and any existing cuts or skin lesions must be properly protected. All procedures creating aerosols to be conducted in a microbiological safety cabinet. A biological agents questionnaire must be completed and returned to occupational health before any work with unfixed human tissue. Users should be hepatitis B vaccinated. In the event of an incident, users must report and seek appropriate medical advice. Occupational health must be informed. Tubes used for unprocessed sections must be disposed of in human waste container to be autoclaved. Samples must be treated with universal precaution.

- Hazardous chemical - QIAzol Lysis Reagent (Phenol, guanidine thiocyanate) 

QIAzol Lysis Reagent is toxic by inhalation, contact with skin and if swallowed. It causes severe burns and serious eye damage. It is toxic to aquatic life. Wear long cuffed nitrile gloves and always use in a chemical fume hood. Do not wash into drains, dispose via chemical waste route. Keep a bottle of PEG 300 in the fume cupboard while working with reagents containing phenol.

A guide for how to treat contact is available attached to fume hood 1. Familiarise yourself with it.

- Hazardous chemical – Chloroform 

Chloroform is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is toxic if inhaled. It is harmful to aquatic life. Wear nitrile gloves and always use in a chemical fume cupboard. Do not wash into drains, dispose via chemical waste route. NB: Chloroform is a reproductive toxicity hazard – not to be used by pregnant people.

- Hazardous chemical – Buffer RLT and RWT (Guanidine thiocyanate) 

Buffer RLT and RW1 are flammable liquids and vapours. They can cause serious eye damage. Wear nitrile gloves and use in a chemical fume cupboard. Keep away from heat, hot surfaces, sparks and other sources of ignition. Do not wash into drains, dispose of via chemical waste route.

- Hazardous chemical – DNase I 

DNase I may cause an allergic reaction or asthma symptoms if inhaled. Wear protective gloves and make up stock from desiccated DNase I in the fume cupboard. Do not wash into drains, dispose via chemical waste route.

- Hazardous material- **Dry ice**

Dry ice can induce asphyxiation or Burns/frostbite. Dry ice will be used for storing the tissue samples. Always wear gloves and use ice scoop when handling dry ice. Store dry ice in a well-ventilated room in a Styrofoam or insulated cooler. Never store dry ice in the cold room or freezer. Do not pour dry ice into sinks.

Before starting:

Prepare working area in fume cupboard:

- Spray down surfaces, pipettes, tip boxes, ice box with RNase Zap and allow to air dry.
- Ensure PEG 300 solution is close by.
- Set centrifuge to 4°C.
- Place DNase I aliquot on ice.
- Make up 70% and 80% ethanol with RNase free H_2O – and molecular grade 100% ethanol. MAKE UP FRESH.

Protocol

1) Collect tissue samples and maintain on dry ice until use.

In fume cupboard:

2) Add 500µl QIAzol Lysis reagent to sample.
   (>1ml per 100mg required, tissue < 10% volume of QIAzol)
3) Homogenise tissue in QIAzol by pipetting up and down with p1000 pipette (keep samples on ice until all have been homogenised).
4) Leave to rest at room temperature (RT) for 5 minutes (min)
5) Add 100µl Chloroform (prime pipette tip by pipetting up and down with chloroform to prevent dripping) securely cap tube before vigorous shaking ~ 15 seconds (sec). NB: Chloroform is a reproductive toxicity hazard – not to be used by pregnant people.
6) Leave to rest at RT for 2-3 min
7) Centrifuge 4°C 15 min 12,000 x g
8) Transfer upper aqueous phase to new tube. Set pipette to 100µl and take as much as possible without disrupting the white middle layer or taking up any QIAzol reagent.
9) Dispose of lower layers in labelled chemical waste bin in fume cupboard
10) Add equal volume (~ 300µl) of 70% Ethanol (made up from 100% molecular grade Ethanol and nuclease free water)
11) Vortex
12) Transfer up to 700µl to RNeasy Mini Elute spin column
13) Centrifuge > 8000 x g 15-20 sec
14) Discard flow through in labelled chemical waste bin in fume cupboard and use a new collection tube
15) Add 350µl RW1 to spin column
16) Centrifuge > 8000 x g 15-20 sec
17) Discard flow through in labelled chemical waste bin in fume cupboard
18) Make up DNase solution 10µl DNase in 70µl RDD per sample (make in excess -10%)
19) Mix by inverting tube **N.B. DNase is fragile do not vortex**
20) Add 80µl DNase mix directly onto spin column
21) Leave at RT for 15min
22) Add 350µl RW1 to spin column

23) Centrifuge > 8000 x g 15-20 sec
24) Discard flow through and collection tube. Transfer spin column to new collection tube.

On lab bench

25) Clean lab bench with RNase Zap
26) Add 500µl RPE: **N.B. RPE comes as concentrate ensure molecular grade Ethanol has been added to buffer before use**
27) Centrifuge > 8000 x g 15-20 sec
28) Discard flow through
29) Add 500µl 80% ethanol (made up from 100% molecular grade ethanol and nuclease free water)
30) Centrifuge > 8000 x g 2 min
31) Discard flow through and collection tube
32) Place RNA spin column in new collection tube
33) RNase Zap the lid and surface area of centrifuge
34) Centrifuge with lids open for 5 min max speed
35) Discard flow through and collection tube
36) Place spin column in new labelled Eppendorf (Sample ID, Region, tissue type, RNA, date, initials) **N.B Label tubes with ethanol resistant marker.**
37) Add 35µl RNase free water directly to spin column membrane, incubate 3-5 min
38) Centrifuge 1 min full speed
39) Pipette any residual liquid remaining in the spin column (e.g. on walls of spin column) and reapply to membrane.
40) Repeat centrifuge 1 min at full speed

Follow up with QC of RNA with nanodrop – use 2µl/ sample and blank with RNase free H_2O and update MAP database with QC metrics for all samples (e.g. RNA concentration, A260/A280, A260/A230, Final volume, Final mass, QC date).

<u>RNA sample submission</u>

A new study should be set up and added into the **IGF_Segstudy_log database.xlsx**

Samples to be loaded vertically (e.g. A1-H1) on a 96 well plate and input on the sample submission form in this order. Ensure sample ID on form matches bioinformaticians format.
Sample submission available from genomics facility. Add foil adhesive cover to plate when finished.
Sample plate: Add all RNA sample to well.
QC plate: Using multichannel, to transfer 5µl of sample from main sample plate to QC plate.

At the Imperial genomics sequencing facility, our requested service:
rRNA depletion + bulk RNA library preparation + sequencing
50 million reads per sample

****Once sequencing has been performed update details in the IGF_Segstudy_log database.xlsx including (e.g., Sequencing machine, library prep method, RDS file path ect...) ****

Accidental exposure/ first aid: In case of skin contact with phenol: Wash skin; keep washing with running COLD water for 2-3 minutes. After the initial irrigation with water, swab / wipe affected area

repeatedly with a solution of Polyethylene Glycol 300 (PEG) until the bottle runs out, frequently changing the swab for a fresh swab soaked with PEG. Then flush with copious amounts of COLD water again for at least 10 min. If necessary, seek medical attention.

For skin contact with chloroform and buffer RW1/RLT: Wash with soap and copious amounts of water. Remove any contaminated clothing or shoes.

First aid treatment for inhalation: Remove person to a well-ventilated area. If person seems to become dizzy or loses consciousness call security and wait for emergency services to arrive.

First aid treatment for eye splash: Rinse thoroughly for at least 15 min using the eye wash station. Seek medical attention.

First aid treatment for ingestion: Do NOT induce vomiting. Rinse mouth with water and seek medical attention immediately.

First aid treatment for cold burn: If possible, place burn in lukewarm water. Clean plastic kitchen film or sterile dry dressings should be used to protect damaged tissues from infection or further injury. If necessary, seek medical attention.

Emergency support through security: 4444 (+442075891000)

In all instances of accident OR near miss, notify the safety department and complete a SALUS report.

https://www.imperial.ac.uk/safety/safety-by-topic/accidents--incidents/

Occupational health contacts: https://www.imperial.ac.uk/occupational-health/

email: occhealth@imperial.ac.uk phone: +44 20 7594 9401

Date	Name	Signature	Trained by	Supervisor