MWAC/UNSW Biospecimen Services

Standard Operating Procedure

Extraction of DNA from Cell Pellet – Isolation of blood genomic DNA/Total RNA

Effective date: 15th May 2023
Review date: 15th May 2026

1. Purpose

The purpose of this document is to describe the procedure for extracting DNA/RNA from a blood cell pellet.

2. Scope

This SOP describes the purification of genomic DNA and total RNA from human blood cells using AllPrep DNA/RNA/miRNA Universal Kit (the Kit) supplied by QIAGEN. This procedure uses blood cells obtained from buffy coat of whole blood. See *Biospecimen Services SOP – Blood processing to cell pellet* listed in *Section 10 - Related Documents*.

This SOP is to be used for the Health Precincts Biobank when DNA/RNA extractions from the blood cell pellet are required. This SOP can be adopted by individual collection protocols for DNA/RNA extractions from blood.

For large scale DNA extractions see *Biospecimen Services SOP - Isolation of genomic DNA from whole blood in large scale* listed in *Section 11 - Related Documents*.

This SOP does not cover isolation of plasma from whole blood, processing blood to a cell pellet, large scale DNA extractions and the uses of DNA and RNA in research.

3. Roles & Responsibilities

The SOP applies to all Biospecimen Services personnel responsible for performing DNA/RNA extractions.

Personnel	Responsibility
Technician	• To ensure that the procedure outlined in this standard operating procedure is closely adhered to, as any changes in the methodology will jeopardise the validity of the results. • To be trained in Good Laboratory Practice and be inducted into the PC2 facility. • Carry out accurate pipetting and avoid cross-contamination of specimens.

4. Materials & Equipment

The materials and equipment listed below are needed to perform this method.

4.1. Equipment

- Microcentrifuge
- Class II Biosafety Cabinet
- Water-bath or heating block

- Vortex
- TissueLyser LT Cat.No.85600
- 4ºC fridge and -80ºC freezer
- P1000, P200 and P10 pipettes
- QIAxpert spectrophotometer

4.2. Consumables

- AllPrep DNA/RNA/miRNA Universal Kit (50) Cat.No.80224
- QIAshredder (50) Cat.No.79654
- Disposable gloves
- Sarstedt 2ml Micro tube, PP, SKIRTED 72.694 tubes
- 1000/1250mL, 200/300µL and 10µL pipette tips with aerosol barriers
- Eppendorf tubes 1.5~1.7mL, sterile
- 5mm diameter stainless steel bead (one/sample) for TissueLyser LT
- Isopropanol
- 100% ethanol
- β-mercaptoethanol (b-ME) for RNA extraction (commercially available solutions are usually 14.3 M)
- QIAxpert Slide-40

5. Safety Requirements

5.1. Clean back-opening gowns and powderless gloves must be worn during all operations in this standard operating procedure.

5.2. All samples must be treated as potential infection risks and must be handled according to good laboratory procedures and methods to prevent occupational exposure.

5.3. Buffer RLT plus in the kit contains guanidine salt, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. The Safety Data Sheet (SDS) for the kit are available online at SDS of AllPrep DNARNAmiRNA Universal Kit

5.4. All solutions must be appropriately labelled following the UNSW Labelling of Hazardous Substances Guideline (document control number HS429).

5.5. Before proceeding with this method the following safety information must be read:

5.5.1. Safety Data Sheet: β-mercaptoethanol

5.5.2. Safety Data Sheet: Isopropanol

5.5.3. Biospecimen Services Risk Assessments and Safe Work Practices relating to this procedure, saved on the DVCR drive

6. Introduction

6.1. Blood cells were obtained from buffy coat of whole blood. See *Biospecimen Services SOP – Blood processing to cell pellet* listed in *Section 11 - Related Documents*.

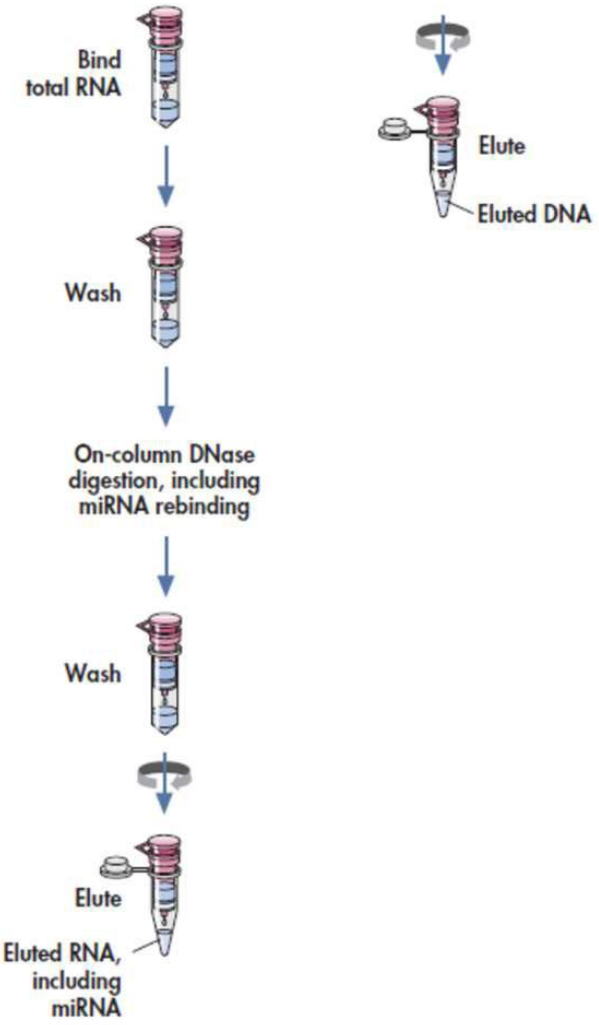
6.2. Book the specimens into OpenSpecimen following the instructions in each individual collection protocol and complete the e-processing form (Appendix B). Concentration, volume and total amount should be recorded in the e-processing form at the end of the Method (Section 7).

6.3. The efficiency of using the Kit is to obtain both gDNA and RNA simultaneous from a single biological sample with pure and maximum yields without the need for toxic substance used in the traditional method.

6.4. The principal of the Kit:

Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate-containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate is then passed through an AllPrep DNA Mini spin column. This column, in combination with the high-salt buffer, allows selective and efficient binding of genomic DNA. On-column Proteinase K digestion in optimized buffer conditions allows purification of high DNA yields from all sample types. The column is then washed and pure, ready-to-use DNA is eluted.

Flow-through from the AllPrep DNA Mini spin column is digested by Proteinase K in the presence of ethanol. This optimized digestion, together with the subsequent addition of further ethanol, allows appropriate binding of total RNA, including miRNA, to the RNeasy Mini spin column. DNase I digestion ensures high-yields of DNA-free RNA. Following DNase I digestion, contaminants are efficiently washed away and high-quality RNA is eluted.



7. Method

The detailed procedure is found in *Simultaneous Purification of Genomic DNA and Total RNA, including miRNA from cells* pages 37 to 47 of **AllPrep DNA/RNA/miRNA Univeral Handbook 11/2020.** Summarised as below:

7.1. Determining the correct amount of starting material as minimum 100 cells and maximum $1x10^6$ cells to make sure that the binding capacity of the spin column is not exceeded as the table below.

Table 1. Spin column specifications

Specification	AllPrep DNA Mini spin column	RNeasy Mini spin column
Maximum binding capacity	100 µg DNA	100 µg RNA
Maximum loading volume	700 µl	700 µl
Nucleic acid size distribution	DNA size of 15–30 kb*	RNA >18 nt
Minimum elution volume	100 µl	30 µl
Maximum amount of starting material:		
Animal cells	1 x 10^7 cells	Entire flow-through from the AllPrep DNA Mini spin column
Animal tissue	30 mg†	Entire flow-through from the AllPrep DNA Mini spin column
Blood	1.5 ml (maximum of 1x10^7 leukocytes)	Entire flow-through from the AllPrep DNA Mini spin column

* Depending on homogenization conditions.

† Do not use more than 20 mg of tissue stabilized in RNAprotect Tissue Reagent or Allprotect Tissue Reagent.

7.2. If using the new *AllPrep DNA/RNA/miRNA Universal Kit* for the first time:

7.2.1. Before using Buffer FRN for the first time, check whether a precipitate has formed. If necessary, dissolve by warming with gentle agitation.

7.2.2. Add 42 ml isopropanol to the bottle containing 14 ml Buffer FRN concentrate. Tick the check box on the bottle label to indicate that isopropanol has been added.

7.2.3. Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µl RNase-free water by injection and gentle inverting the vial without vortex. Remove the stock solution from the vial, divide it into single-use aliquots, and store at –30°C to –15°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

7.2.4. Preparing Buffer RPE by adding 4 volumes (44 ml) ethanol (96–100%) to the bottle containing 11 ml Buffer RPE concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Before starting the procedure, mix reconstituted Buffer RPE by shaking.

7.2.5. Preparing Buffer AW1 by adding 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Before starting the procedure, mix reconstituted Buffer AW1 by shaking.

7.2.6. Preparing Buffer AW2 by adding 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

7.3. Important points before starting:

7.3.1. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube.

7.3.2. Homogenised cell lysates can be stored at –70ºC for several months. Frozen lysates should be incubated at 37ºC in a water bath or heating block until completely thawed and salts are dissolved.

7.3.3. β-mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use for RNA extraction. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus is stable at room temperature (15–25ºC) for 1 month after addition of β-ME.

7.3.4. Buffer RLT Plus, Buffer FRN and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach.

7.3.5. Perform all steps of the procedure at room temperature. During the procedure, work quickly. Ensure that the centrifuge does not cool below 20ºC.

7.4. Procedure

7.4.1. **Separate for RNA and DNA processing**

- Thaw frozen blood cell pellet (no more than 1 x 10^7 cells) in the 2 ml microtube at 37ºC in a water bath or heat block.
- Centrifuge pellet for 5 min at 300 x g and remove all supernatant.
- Add 600 µl Buffer RLT Plus, vortex for 1 min. For better homogenising the lysate, either (a) pipet the lysate into a QIAshredder in a 2 ml collection tube and centrifuge for 2 min at max speed or (b) add a steel bead into a sample tube and put into TissueLyser LT for 2 min at 50Hz operation.
- Briefly centrifuge the tube to reduce foam.
- Transfer the homogenised lysate to an AllPrep DNA mini spin column placed in a 2 ml collection tube and centrifuge for 1 min at max speed.
- Place the DNA mini column in a new 2 ml collection tube and store at room temperature or at 4ºC for purification later.
- Transfer the flow-through to a new 2 ml microtube for RNA purification.

7.4.2. **Total RNA (including miRNA) purification**

- Add 80 µl Proteinase K to the flow-through and mix by pipetting.
- Add 350 µl of 100% ethanol and mix well (don't centrifuge).
- Incubate 10 min at room temperature.
- Add 750 µl of 100% ethanol and mix well (don't centrifuge).
- Transfer up to 700 µl of sample to an RNeasy mini spin column placed in a 2 ml collection tube. Centrifuge for 15 sec at max speed and discard the flow-through for reusing the collection tube.
- Repeat above till the entire sample has passed the spin column. Discard the flow-through before reusing the collection tube.
- Add 500 µl Buffer RPE to the spin column, centrifuge for 15 sec at max speed. Discard the flow-through before reusing the collection tube.
- Add 10 µl DNase I stock solution to 70 µl Buffer RDD. GENTLY inverting the tube and centrifuge briefly.
- Add the DNase I incubation mix 80 µl directly onto the spin column membrane and incubate 20ºC-30ºC for 15 min.
- Add 500 µl Buffer FRN to the spin column, centrifuge 15 sec at max speed. Save the flow-through.

- Place the spin column in a new 2 ml collection tube. Apply the flow-through from the previous step to the spin column. Centrifuge 15 sec at max speed. Discard the flow-through before reusing the collection tube.
- Add 500 µl Buffer RPE to the spin column. Centrifuge 15 sec at max speed. Discard the flow-through before reusing the collection tube.
- Add 500 µl of 100% ethanol to the spin column, centrifuge 2 min at max speed to wash the spin column membrane. To eliminate possible carryover of ethanol, place the spin column in a new 2 ml collection tube, centrifuge 2 min at max speed.
- Place the spin column in a new supplied 1.5 ml RNase-free collection tube. Add 30-50 µl RNase-free water to the spin column membrane and centrifuge for 1 min at 10000 rpm to elute the RNA.
    - Repeat the centrifuge with another 30-50 µl RNase-free water into the same 1.5 ml collection tube for higher yield,

      OR
    - Repeat the centrifuge using the eluate into the same 1.5 ml collection tube for higher concentration.

7.4.3. **Genomic DNA purification**

- Add 350 µl Buffer AW1 to the DNA spin column from Step (7.4.1). Centrifuge 15 sec at max speed. Discard the flow-through and re-use the collection tube.
- Add 20 µl Proteinase K to 60 µl Buffer AW1, mix gently, apply the mixture to the DNA spin column membrane.
- Incubate for 5 min at room temperature.
- Add 350 µl Buffer AW1 to the spin column, centrifuge 15 sec at max speed. Discard the flow-through before collection tube reuse.
- Add 500 µl Buffer AW2 to the spin column, centrifuge 2 min at max speed. Spin another 1 min if the column is in contact with the flow-through.
- Place the spin column in a new 1.5 ml collection tube. Add 100 µl Buffer EB to the column membrane. Incubate at room temperature for 1 min and centrifuge 1 min at 10000 rpm to elute the DNA.
- Repeat this step to further elute DNA. For higher DNA concentration, elute with 2x50 µl Buffer EB.

7.5. Quantitate DNA/RNA using QIAxpert spectrophotometer

7.5.1. See the QIAxpert User Manual for Operation of QIAxpert Spectrophotometer.

7.5.2. DNA in the range 100 - 500 ng/µl is preferred, dilute with 1x TE if required.

7.6. Label and store DNA at assigned spot in -80ºC freezer

7.6.1. To assign a storage location, when booking in the specimen, choose the current box, this will be in the e-processing form (Appendix B) and the system will select the next available spot when the **Submit** button is clicked in OpenSpecimen.

7.6.2. Specimens should be labelled with, at a minimum, specimen number, PPID, specimen type, box/spot location, Collection Protocol. See *Biospecimen Services SOP – Biospecimen Storage and Retrieval* listed in *Related Documents – Section 11* for more information.

7.6.3. To label the DNA tube, refer to file LTB-WI-006-V3 Print Permanent label from LabelMark5.pdf

8. Shutdown

8.1. Discard solid waste into Bio-waste Bin and liquid waste into Betadine antiseptic solution containing bottle according to Laboratory Hazardous Waste Disposal Guideline.

8.2. Make sure all bench areas are clean and reagents are stored appropriately.

8.3. Swab down the entire work surface of the biosafety cabinet and working bench with 80% v/v.

8.4. Switch off biosafety cabinet and other instruments.

9. Monitoring of compliance to this SOP is ongoing.

9.1. The Biospecimen Services Manager or their delegate is responsible for ongoing monitoring of biobank operations to verify compliance with this SOP.

9.2. The Biospecimen Services Manager or their delegate is responsible for obtaining annual updates to this SOP and for communicating these changes to all personnel.

10. Definitions

Term or Abbreviation	Definition
Buffer FRN	Buffer made by QIAGEN used in DNA/RNA extractions. Main components are guanidine thiocyanate and isopropanol.
Buffer RPE	Buffer for washing membrane-bound RNA made by QIAGEN.
Buffer AW1	Wash buffer made by QIAGEN.
Buffer AW2	Wash buffer made by QIAGEN.
Buffer RLT Plus	Lysis buffer for lysing cells and tissue before RNA/DNA isolation. Made by QIAGEN.
Buffer RDD	Buffer RDD is a component of the QIAGEN RNase-Free DNase Set, which can be used in combination with most RNeasy Kits. Buffer RDD provides efficient on-column digestion of DNA and also ensures that the RNA remains bound to the column.
TE	The most common buffer used for dilution and storage of DNA. TE buffer is made of Tris-Chloride (10 mM) EDTA (1 mM) and has pH8.0.
Buffer EB	Elution buffer used in QIAGEN kits.
PPID	Unique code or number assigned to the participant.
Specimen Number	Unique code or number assigned to individual specimens.
Collection Protocol	A Collection Protocol is an ongoing accrual and storage of specimens, undertaken by a Principal Investigator (PI) in association with Biospecimen Services. A Collection Protocol in OpenSpecimen has defined specimen collection groups, time points and a predetermined specimen type and processing protocol.

11. Related Documents

Document	Description
Biospecimen Services SOP – Blood Processing to Cell Pellet, Version 2, 15/05/2023	SOP that describes how to make a cell pellet form whole blood.
Biospecimen Services SOP – Extraction of DNA from a Cell Pellet - Isolation of genomic DNA from whole blood in large scale, Version 1, 15/05/2023	SOP that describes how to extract DNA from cell pellet made from whole blood using the QIASymphony. This is for large scale DNA extractions.

Biospecimen Services SOP – Biospecimen Storage and Retrieval, Version 3, 12/05/2023	SOP that describes how to store, retrieve and ship biospecimens.
AllPrep DNA/RNA/miRNA Universal Handbook 11/2020	Handbook from QIAGEN that details the procedure to follow when using the AllPrep DNA/RNA/miRNA Universal Kit
QIAxpert User Manual 11/2021	User Manual for the QIAxpert
NSW/CTRNet Required Operational Practice 9: Biospecimen Collection and Processing	ROP that describes the key principles regarding biospecimen collection and processing that should be adhered to, to meet the current best practice standards.
NSW/CTRNet Required Operational Practice 10: Biospecimen Storage and Retrieval	ROP that describes the key principles regarding biospecimen storage and retrieval that should be adhered to, to meet the current best practice standards.
NSW/CTRNet Required Operational Practice 13: Safety and Waste Disposal	ROP that describes the key principles regarding safety and waste disposal that should be adhered to, to meet the current best practice standards.
UNSW Labelling of Hazardous Chemicals Guideline (document control number HS429).	UNSW Guideline for labelling hazardous substances in laboratories.
UNSW Laboratory Hazardous Waste Disposal	UNSW Guideline for disposing of hazardous waste in laboratories.
UNSW Biosafety Policy	UNSW Policy for identifying biohazardous material and meeting legislative and regulatory requirements.
UNSW Personal Protective Equipment Guideline	UNSW Guideline for selecting, using and maintaining PPE.

12. Appendices

12.1. Appendix A: Example labels

12.2. Appendix B: e-Processing Form

13. References, Regulations & Guidelines

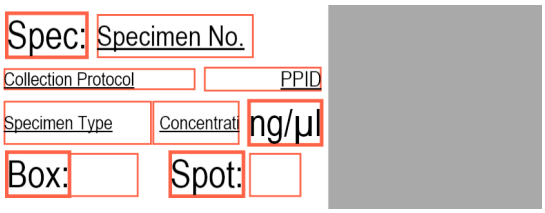
13.1. QIAGEN (November 2020). *Simultaneous Purification of Genomic DNA and Total RNA, including miRNA from cells*, pages 37 to 47, **AllPrep DNA/RNA/miRNA Universal Handbook 11/2020**

14. Version History & Authorisation

Version	Date	Author	Summary of Changes	Authorised By:
1	15/05/2023	Pearl Zhu/ Ussha Pillai	Original Document	Manager, Biospecimen Services: Anusha Hettiaratchi

Appendix A: Example labels

1. Template Label



Appendix B: e-Processing Form