MWAC/UNSW Biospecimen Services

Standard Operating Procedure

Isolation of DNA/total RNA from Fresh/Frozen Tissue

Effective date: 16/05/2023
Review date: 16/05/2026

1. Purpose

The purpose of this document is to describe the procedure for isolating DNA/RNA from fresh and frozen tissue.

2. Scope

This SOP describes the purification of DNA and total RNA from human fresh and frozen tissue using *AllPrep DNA/RNA/miRNA Universal Kit (the Kit)* supplied by QIAGEN. This procedure uses human tissue obtained during routine surgical procedures. See *Biospecimen Services SOP – Fresh Tissue Processing* listed in *Section 11 - Related Documents*.

This SOP is to be used for the Health Precincts Biobank when DNA/RNA extractions from fresh and frozen tissue are required. This SOP can be adopted by individual collection protocols for DNA/RNA extractions from tissue.

This SOP does not cover extraction of genomic DNA and total RNA from blood and the uses of DNA and RNA in research.

3. Roles & Responsibilities

The SOP applies to all Biospecimen Services personnel responsible for processing biospecimens.

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
- Biological Safety Cabinet Class II
- Water-bath or heating block
- Vortex

- TissueLyser LT Cat.No.85600
- Fridge and 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 (optional)
- 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 Cat.No69989 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*, *Buffer FRN* and *Buffer AW1* in the kit contain 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 Chemicals 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

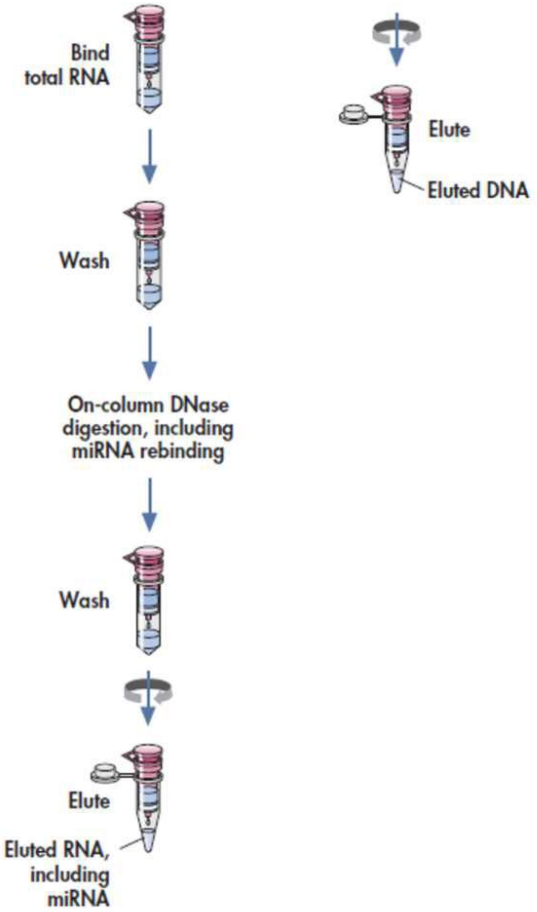
6.1. The efficiency of using *the Kit* is to obtain both DNA and RNA simultaneous from a single biological sample with pure and maximum yields without the need for toxic substances (e.g. phenol used in traditional DNA/RNA extraction methods).

6.2. 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 optimised 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 Tissues* pages 26 to 36 of AllPrep DNA/RNA/miRNA Universal Handbook 11/2020. Summarised as below:

7.1. 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)

7.2. Determine the correct amount of starting material as 30mg for fresh tissue and 20mg for frozen tissue that has been stabilised with RNA*later* to make sure that the binding capacity of the spin column is not exceeded. Maximum capacities are listed in 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.

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

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

7.3.2. 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.

7.3.3. 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.

7.3.4. 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.

7.3.5. 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.

7.3.6. 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.

7.4. Important points before starting.

7.4.1. Frozen tissues should be thawed slightly so that they can be dislodged by flicking the tube.

7.4.2. Homogenized tissue lysates can be stored at –70ºC for several months. Incubate frozen lysates at 37ºC in a water bath/heat block until completely thawed and salts are dissolved. Prolonged incubation may compromise RNA integrity.

7.4.3. β-mercaptoethanol (β-ME) must be added to *Buffer RLT Plus* before use. 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.

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

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

7.5. Procedure

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

(a) Remove tissue from RNA*later*/reagent using forceps. Determine the amount of tissue. Do not use more than 20mg of stabilised tissue.

(b) Cut tissues as small as possible. Put them into a 2ml tube.

(c) Add 600µl *Buffer RLT Plus*, vortex for 1 min. Add a frozen 5mm stainless steel bead. Disruption is carried out using the TissueLyser LT for 5min at 50Hz.

(d) Optional: Briefly centrifuge the tube to reduce foam.

(e) Transfer the homogenised lysate to an AllPrep DNA mini spin column placed in a 2ml collection tube and centrifuge for 1min at max speed.

(f) Place the DNA mini column in a new 2ml collection tube and store at room temperature or at 4°C for purification later. Transfer the flow-through to a new 2ml microtube for RNA purification.

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

(g) For fatty tissues (e.g. fat, brain, breast), add 150µl chloroform to the flow-through from step (f), vortex thoroughly. Centrifuge at 4°C for 3min at max speed. Transfer the aqueous phase to a new 2ml microcentrifuge tube.

(h) Add 80µl *Proteinase K* to the flow-through from step 6 or the aqueous phase from step (g) and mix by pipetting.

(i) Add 350µl of 100% ethanol and mix well (don't centrifuge).

(j) Incubate 10min at room temperature.

(k) Add 750µl of 100% ethanol and mix well (don't centrifuge).

(l) Transfer up to 700µl of sample to a *RNeasy mini spin column* placed in a 2ml collection tube. Centrifuge for 15s at max speed and discard the flow-through for reusing the collection tube.

(m) Repeat step (l) until the entire sample has passed the spin column. Discard the flow-through for reusing the collection tube again.

(n) Add 500µl *Buffer RPE* to the spin column, centrifuge for 15s at max speed. Discard the flow-through for reusing the collection tube.

(o) Add 10µl *DNase I* stock solution to 70µl *Buffer RDD*. GENTLY inverting the tube and centrifuge briefly.

(p) Add the *DNase I incubation mix* 80µl directly onto the spin column membrane and incubate 20°C-30°C for 15 min.

(q) Add 500µl *Buffer FRN* to the spin column, centrifuge 15s at max speed. Save the flow-through for use in step (r).

(r) Place the spin column in a new 2ml collection tube. Apply the flow-through from the step before to the spin column. Centrifuge 15s at max speed. Discard the flow-through. Reuse the collection tube in step (s).

(s) Add 500µl *Buffer RPE* to the spin column. Centrifuge 15s at max speed. Discard the flow-through. Reuse the collection tube in step (t).

(t) Add 500µl of 100% ethanol to the spin column, centrifuge 2min at max speed to wash the spin column membrane. To eliminate possible carryover of ethanol, place the spin column in a new 2ml collection tube, centrifuge 2min at max speed.

(u) **Optional**: Place the *RNeasy Mini spin column* in a new 2 ml collection tube (supplied) and discard the old collection tube with the flow-through. Centrifuge at max speed for 2 min.

(v) Carefully remove the spin column and place in a new supplied 1.5ml RNase-free collection tube. Add 30-50µl RNase-free water to the spin column membrane and centrifuge for 1min at 10,000rpm to elute the RNA.

(w) If the expected RNA yield is >30µg;

- Repeat the centrifuge with another 30-50µl RNase-free water into the same 1.5ml collection tube for **higher yield**

  OR

- Repeat the centrifuge using the eluate into the same 1.5ml collection tube for **higher concentration** but 15-30% less yield.

7.5.3. **Genomic DNA purification**

(x) Add 350µl *Buffer AW1* to the DNA spin column from Step (f). Centrifuge 15s at max speed. Discard the flow-through. Reuse the collection tube in step (y).

(y) Add 20µl *Proteinase K* to 60µl *Buffer AW1*, mix gently, apply the mixture to the DNA spin column membrane.

(z) Incubate for 5min at room temperature.

(aa) Add 350µl *Buffer AW1* to the spin column, centrifuge 15s at max speed. Discard the flow-through. Reuse the collection tube in step (bb).

(bb) Add 500µl *Buffer AW2* to the DNA spin column, centrifuge 2min at max speed. Discard the flow-through. Spin another 1min if the column is in contact with the flow-through.

(cc) Place the DNA spin column in a supplied new 1.5ml collection tube. Add 100µl *Buffer EB* to the column membrane. Incubate at room temperature for 1min and centrifuge 1min at 10,000rpm to elute the DNA.

(dd) Repeat step (cc) to further elute DNA. For higher DNA concentration, elute with 2x50µl *Buffer EB* but yield may be reduced.

7.6. Quantitate DNA/RNA using QIAxpert spectrophotometer

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

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

7.7. Label and store RNA and DNA in assigned spot in -80^{o}C freezer

7.7.1. 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.

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

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

8. Process 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 ethanol.

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.
RNA*Later*	RNAlater is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissue to stabilize and protect cellular RNA in situ in unfrozen specimens.
Kunitz unit	A Kunitz unit is used to measure DNAse activity.
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 – Fresh Tissue Processing, Version 2, 16/05/2023	SOP that describes how to process and store fresh tissue samples.
Biospecimen Services SOP – Biospecimen Storage and Retrieval, Version 3, 12/05/2023	SOP that describes how to store, retrieve and ship biospecimens.
LTB-WI-006-V3 Print Permanent label from LabelMark5	Instructions on how to print labels saved on the DVCR drive.

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 Procedure	UNSW Procedure 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

Appendix A: Example labels

Appendix B: e-Processing Form

13. References, Regulations & Guidelines

12.1. QIAGEN (2020). *Simultaneous Purification of Genomic DNA and Total RNA, including miRNA from Tissues* pages 26 to 36 AllPrep DNA/RNA/miRNA Universal Handbook 11/2020.

12.2. SigmaAldrich (2022) RNA*Later* description RNAlater Storage Reagent for RNA protection Sigma (sigmaaldrich.com)

12.3. International Society for Biological and Environmental Repositories (ISBER) (2018) Best Practices: Recommendations for Repositories, Fourth Edition ISBER Best Practices For Repositories - ISBER

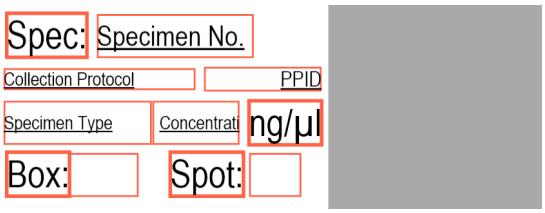
14. Version History & Authorisation

Version	Date	Author	Summary of Changes	Authorised By:
1	16/05/2023	Pearl Zhu/ Ussha Pillai	Original	Manager, Biospecimen Services: Anusha Hettiaratchi

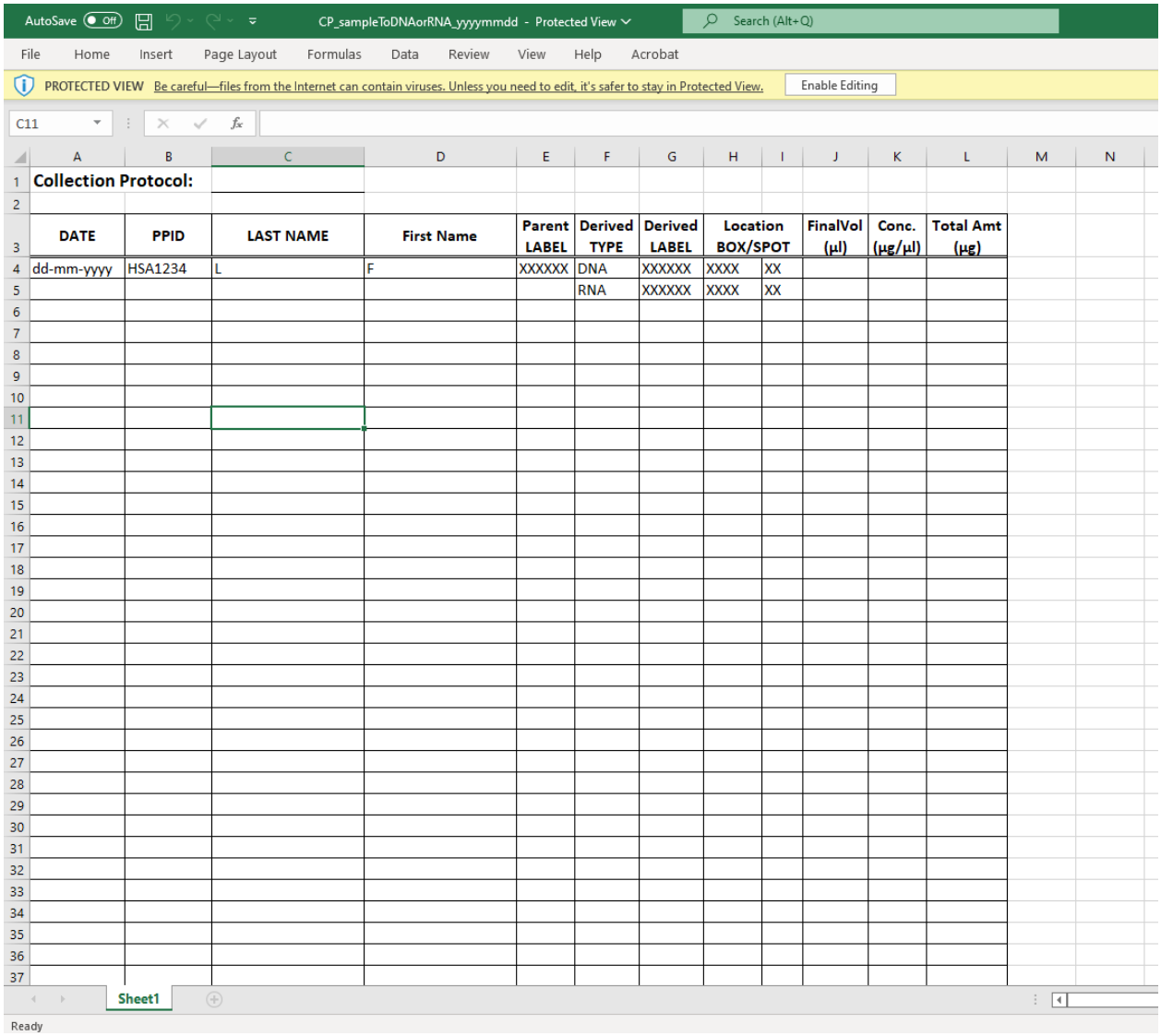
Appendices

Appendix A: Example labels

1. Template Label



Appendix B: e-Processing Form



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PROTECTED VIEW Be careful—files from the Internet can contain viruses. Unless you need to edit, it's safer to stay in Protected View. Enable Editing

C11

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	**Collection Protocol:**													
2														
3	**DATE**	**PPID**	**LAST NAME**	**First Name**	**Parent LABEL**	**Derived TYPE**	**Derived LABEL**	**Location BOX/SPOT**		**FinalVol (µl)**	**Conc. (µg/µl)**	**Total Amt (µg)**		
4	dd-mm-yyyy	HSA1234	L	F	XXXXXX	DNA	XXXXXX	XXXX	XX					
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Sheet1

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