



VERSION 3
AUG 02, 2023

OPEN  ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.kxygx3mrwg8j/v3

Protocol Citation: Annika Fendler 2023. DNA/RNA extraction from fresh-frozen tissue, AllPrep DNA/RNA/miRNA Universal Kit. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kxygx3mrwg8j/v3>
Version created by [Annika Fendler](#)

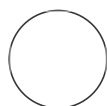
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DNA/RNA extraction from fresh-frozen tissue, AllPrep DNA/RNA/miRNA Universal Kit V.3

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ABSTRACT

Protocol for combined RNA and DNA extraction from fresh-frozen tissue using the AllPrep DNA/RNA/miRNA Universal Kit.

GUIDELINES

DNase I stocks can be used 4 weeks after being thawed but should not be frozen again.

Never thaw tissue when processing.

Always use a fresh scalpel and plate when cutting tissues to prevent cross contamination.

Protocol status: Working
We use this protocol and it's working

Created: Aug 02, 2023

Last Modified: Aug 02, 2023


PROTOCOL integer ID:
85855


Keywords: DNA, RNA, Fresh-frozen tissue, Qiagen AllPrep


MATERIALS


 AllPrep DNA/RNA/miRNA Universal Kit (50) Qiagen Catalog #80224

 Genomic DNA ScreenTape Agilent Technologies Catalog #5067-5365

 Qubit™ dsDNA BR Assay Kit Thermo Fisher Scientific Catalog #Q32853

 Qubit RNA BR Assay Kit Thermo Fisher Scientific Catalog #Q10211

 RNA ScreenTape and Reagents Agilent Technologies

 EB buffer Qiagen Catalog #19086

β-ME

EtOH

Isoprop

1.5 and 2 ml LoBind tubes

TissueLyser Beads 5 mm

SAFETY WARNINGS



All steps with β-ME should be done under the hood.
Reduce use as sharps when working with primary tissue and notify Bettina Ergün in case of an accident.

ETHICS STATEMENT

Primary patient tissue can only be used after appropriate ethics approval has been obtained. Only use tissue for which an EVE has been signed and which has been approved to be used for the study you are working on.

BEFORE START INSTRUCTIONS

Preparations:

FRN buffer: Add 42 ml Isoprop to new bottle


RPE buffer: Add 44 ml EtOH to new bottle




AW1 buffer: Add 25 ml EtOH to new bottle






AW2 buffer: Add 30 ml EtOH

DNase I stocks: 550 µl RNase-free water to lyophilised DNase I, aliquot and store at -20°C for 9 months)


Prepare Working Solutions

- 1 From  RNase-Free DNase Set Qiagen Catalog #79254

DNase I working solution:  70 µL RDD +  10 µL DNase I working solution per sample
- 2 Included within  AllPrep DNA/RNA/miRNA Universal Kit (50) Qiagen Catalog #80224

Proteinase K working solution for DNA isolation:  60 µL AW1 +  20 µL Proteinase K per sample
- 3 Included within  AllPrep DNA/RNA/miRNA Universal Kit (50) Qiagen Catalog #80224
Add  10 µL β-Mercaptoethanol (not included in kit) per  1 mL of RLT plus buffer

Tissue preparation

- 4 This protocol is for  Sample





- 4.1 Optional: Weigh tissue before start to decide for volume

4.2 Enter a complete list of samples used for each experiment below:

SampleID	Type (TURB, T1, T2)	Optional: Weight (mg)	Comment	DNA (ng/ul)

List of tissue sample used in experiment

- 5 Keep tubes with tissue on dry ice and make sure that they do not thaw during processing. Transfer the tissue piece into a 6-well plate or small petry dish placed on dry ice and cut an appropriate piece of tissue (ca. 10-30 mg) with a safety scalpel.

- 6 Transfer tissue in  350 µL for up to 10 mg of tissue
-  600 µL for 10 to 30 mg or stabilised tissue
- RLT + β-ME in 2 ml DNA LoBind tube.

- 7 Add a 5mm bead to each tube and lyse tissue in TissueLyser for  00:02:00 @ 20 Hz

2m

Equipment

TissueLyser II

NAME

Bead Mill

TYPE

QIAGEN









BRAND

85300



SKU
















<https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissueLyser-ii/#orderinginformation>

LINK










- 8 Spin down for  00:01:00 @  9000 x g 1m
- 9 Turn tube rack and lyse tissue for  00:02:00 @ 20 Hz 2m
- 10 Spin down for  00:01:00 @  9000 x g 1m
- 11 Add lysed product to DNA Mini Spin Column
- 12 Spin  00:00:30 @  max x g, repeat if any liquid remains on column 30s
- 13 Transfer column to a new collection tube and store tube at  4 °C until DNA extraction
- 14 Transfer flow-through to new 2 ml LoBind tube

RNA extraction

- 15 Add  50 µL for up to 10 mg tissue  80 µL for 10 to 30 mg or stabilised tissue Proteinase K
(not diluted), mix by pipetting

- 16 Add  200 µL for up to 10 mg tissue  350 µL for 10 to 30 mg or stabilised tissue EtOH abs., mix by inverting, spin down liquid
- 17 Incubate  00:10:00 @  Room temperature 10m
- 18 Add  400 µL for up to 10 mg tissue  750 µL for 10 to 30 mg or stabilised tissue EtOH abs. , mix by pipetting
- 19 Add  700 µL to RNeasy spin column and spin for  00:00:30 @  max x g and discard 30s
flow-through
- 20 Repeat until all liquid passed through the column
- 21 Add  500 µL RPE
- 22 Spin  00:00:30 @  max x g and discard flow-through 30s
- 23 Add  80 µL DNase I working solution directly onto the membrane and incubate 15m
 00:15:00 at  Room temperature

- 24 Add  500 μ L FRN
- 25 Spin  00:00:30 @  max x g and ***don't*** discard flow-through and place column in new collection tube 30s
- 26 Add flow-through again to column
- 27 Spin  00:00:30 @  max x g and discard flow-through 30s
- 28 Add  500 μ L RPE
- 29 Spin  00:00:30 @  max x g and discard flow-through 30s
- 30 Add  500 μ L EtOH abs
- 31 Spin  00:02:00 @  max x g and place column in new collection tube 2m

- 32 Spin  00:02:00 @  max x g and place column in new 1.5 ml collection tube 2m
- 33 Add  30 µL of RNase-free H₂O
- 34 Incubate  00:01:00 @  Room temperature 1m
- 35 Spin down  00:01:00 @  8000 x g 1m
- 36 QC: Qubit  Qubit RNA BR Assay Kit Thermo Fisher Scientific Catalog #Q10211 and tape station  RNA ScreenTape and Reagents Agilent Technologies

36.1

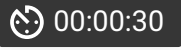

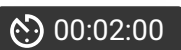
Note









Upload results from Tapestation here

DNA extraction

11m

- 37  350 µL AW1 auf DNA Mini Spin Column geben

- 38 Spin  00:00:30 @  max x g and discard flow-through 30s
- 39  80 μ L Proteinase K working solution directly onto membrane
- 40 Incubate  00:05:00 @  Room temperature 5m
- 41 Add  350 μ L AW1
- 42 Spin  00:00:30 @  max x g and discard flow-through 30s
- 43 Add  350 μ L AW2
- 44 Spin  00:02:00 @  max x g and place column in new 2 ml collection tube 2m
- 45 Spin  00:01:00 @  max x g and place column in new 1.5 ml collection tube 1m

- 46 Add  50 μ L  EB buffer Qiagen Catalog #19086 directly onto membrane
- 47 Incubate  00:01:00 @  Room temperature 1m
- 48 Spin down  00:01:00 @  8000 x g 1m
- 49 QC: Qubit DNA  Qubit™ dsDNA BR Assay Kit Thermo Fisher Scientific Catalog #Q32853
and tape station  Genomic DNA ScreenTape Agilent Technologies Catalog #5067-5365

49.1

Note

Upload Tapestation results as pdf here.