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Version 1

DNA/RNA extraction from fresh-frozen tissue, AllPrep DNA/RNA/miRNA Universal Kit V.1

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Annika Fendler¹

¹Charite



Annika Fendler

Charite - Universitätsmedizin

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

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

Protocol materials

⊗ 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**

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



Safety warnings

❗ Dnase I stocks can be used 4 weeks after being thawed but should not be frozen again

Before start

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)

Immediately

DNase I: 70 µl RDD + 10 µl DNase I per sample

Proteinase K: 60 µl AW1 + 20 µl Proteinase K per sample



Tissue preparation

1 This protocol is for Sample

Optional: Weigh tissue

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

	SampleID	Optional Weight (mg)	Comment

List of tissue sample used in experiment

2 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.

Make sure that the tissue does not defrost.

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

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

4 Spin down for 00:01:00 @ 9000 x g



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

RNA extraction

- 11 Add 50 µL for up to 10 mg tissue 80 µL for 10 to 30 mg or stabilised tissue
Proteinase K, mix by pipetting
- 12 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
- 13 Incubate 00:10:00 @ Room temperature 10m
- 14 Add 400 µL for up to 10 mg tissue 750 µL for 10 to 30 mg or stabilised tissue
EtOH abs. , mix by pipetting
- 15 Add 700 µL to RNeasy spin column and spin for 00:00:30 @ max x g 30s
and discard flow-through
- 16 Repeat until all liquid passed through the column



- 17 Add  500 μ L RPE
- 18 Spin  00:00:30 @  max x g and discard flow-through 30s
- 19 Add  80 μ L DNase I working solution directly onto the membrane and incubate  00:15:00 at  Room temperature 15m
- 20 Add  500 μ L FRN
- 21 Spin  00:00:30 @  max x g and ***don't*** discard flow-through 30s
- 22 Add flow-through again to column
- 23 Spin  00:00:30 @  max x g and discard flow-through 30s
- 24 Add  500 μ L RPE
- 25 Spin  00:00:30 @  max x g and discard flow-through 30s
- 26 Add  500 μ L EtOH abs
- 27 Spin  00:02:00 @  max x g and place column in new collection tube 2m
- 28 Spin  00:02:00 @  max x g and place column in new 1.5 ml collection tube 2m
- 29 Add  30 μ L of RNase-free H₂O

30 Incubate ⌚ 00:01:00 @ 🌡 Room temperature 1m

31 Spin down ⌚ 00:01:00 @ 🌀 8000 x g 1m

32 QC: Qubit ☒ Qubit RNA BR Assay Kit **Thermo Fisher Scientific Catalog #Q10211** and tape station ☒ RNA ScreenTape and Reagents **Agilent Technologies**

32.1

Expected result

	SampleID	Eluted Volume (µl)	Concentration (ng/ul)	Total mass (ng)	Storage Location

Qubit Results RNA

Expected result

Upload PDF from Tapestation here

DNA extraction

11m

33 🧪 350 µL AW1 auf DNA Mini Spin Column geben

34 Spin ⌚ 00:00:30 @ 🌀 max x g and discard flow-through 30s

35 🧪 80 µL Proteinase K working solution directly onto membrane



- 36 Incubate 00:05:00 @ Room temperature 5m
- 37 Add 350 μ L AW1
- 38 Spin 00:00:30 @ max x g and discard flow-through 30s
- 39 Add 350 μ L AW2
- 40 Spin 00:02:00 @ max x g and place column in new 2 ml collection tube 2m
- 41 Spin 00:01:00 @ max x g and place column in new 1.5 ml collection tube 1m
- 42 Add 50 μ L EB buffer **Qiagen Catalog #19086** directly onto membrane
- 43 Incubate 00:01:00 @ Room temperature 1m
- 44 Spin down 00:01:00 @ 8000 x g 1m
- 45 QC: Qubit DNA
 Qubit™ dsDNA BR Assay Kit **Thermo Fisher Scientific Catalog #Q32853** and tape
station Genomic DNA ScreenTape **Agilent Technologies Catalog #5067-5365**
- 45.1



Expected result

	Sample ID	Eluted Volume (μl)	Concentration (ng/μl)	Total Mass (ng)	Storage Location

Results from Qubit DNA

Note

Upload Tapestation results as pdf here