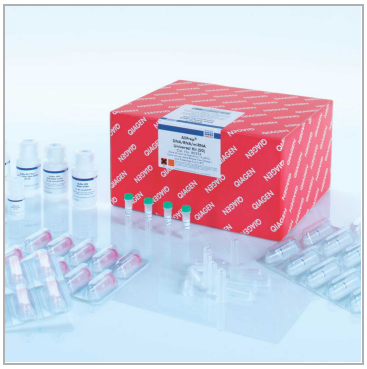

VERSION 2

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Protocol status: Working Protocol has been established in housewith minor changes from original protocol

Created: Jul 03, 2023

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PROTOCOL integer ID: 84402

DNA/RNA extraction from fresh-frozen tissue, AllPrep DNA/RNA/miRNA Universal Kit V.2

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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**

ß-ME

EtOH

Isoprop

1.5 and 2 ml LoBind tubes

TissueLyser Beads 5 mm

SAFETY WARNINGS

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

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

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)

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	Optionel Weigth (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 + ß-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 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

4 Spin down for 00:01:00 @ 9000 x g 1m

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 and discard flow-through 30s

16 Repeat until all liquid passed through the column

17 Add 500 µL RPE

18 Spin 00:00:30 @ max x g and discard flow-through 30s

19 Add 80 µL DNase I working solution directly onto the membrane and incubate 00:15:00 at Room temperature 15m

20 Add 500 µL 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 µL RPE

25 Spin 00:00:30 @ max x g and discard flow-through 30s

26 Add 500 µL 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 µL of RNase-free H_2O

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 µL AW1

38 Spin 00:00:30 @ max x g and discard flow-through 30s

39 Add 350 µL 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 µL 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