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UABMC - Norgen Animal Tissue RNA Purification Protocol

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1 Works for me This protocol is published without a DOI.

NCI PDMC consortium

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ABSTRACT

Purpose

This procedure describes the purification of RNA from snap frozen animal tissues for downstream NGS and other molecular assays.

Scope

The protocol from the Norgen Animal Tissue RNA Purification Kit describes the procedure for isolating RNA from fresh or snap frozen tissue samples. The primary use for this RNA will be for NGS and array based assays.

U01 Subgroup:
(Informatics, neurospheres, microtumors, mouse models, RNA-Seq)

Approvers:
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Responsibilities:
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PROTOCOL CITATION

Christopher Willey 2020. UABMC - Norgen Animal Tissue RNA Purification Protocol. **protocols.io** https://protocols.io/view/uabmc-norgen-animal-tissue-rna-purification-protoc-bg93jz8n

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GUIDELINES

PDMC – Patient-derived model of cancer
BSL2 – Biosafety Level 2
NGS – Next Generation Sequencing
RNA-seq – RNA-sequencing
SF – Snap Frozen

MATERIALS TEXT

For RNA Purification

Tissue:Flash frozen solid tumor or tissue piece at least 8mm^3 (i.e. 2mmx2mmx2mm). Maximum input is 10mg of tissue.
Buffer RL – 30mL
RNA-ase Free Water – 40mL
Wash Solution A – 38mL
Enzyme Incubation Buffer A – 6mL
Elution Solution A – 6mL
Proteinase K – 2 vials : Proteinase should be stored at -20ºC upon arrival
Reconstitute each vial with 600µL of molecular grade (RNase-free) water. **Optional**: Sub aliquot into 200µL fractions and freeze (each sub aliquot good for 10 reactions)
DNase I – 1 vial : DNase I should be stored at -20ºC upon arrival and after reconstitution
Spin Columns – 50
Collection Tubes – 100
Elution Tubes (1.7mL) – 50
Benchtop Microcentrifuge 14,000 x g (~ 14,000 RPM)
96-100% Molecular Grade Ethanol - 500 µL per reaction/sample
Liquid Nitrogen
Mortar and Pestle
Sterile 25 Gauge Needle(s) and Syringe(s)
RNase-free 2mL Microcentrifuge Tubes

SAFETY WARNINGS

1. All fresh human tumors are handled under BSL2 conditions. Work is conducted in the BSC using personal protective equipment and avoiding the use of sharps where possible.
2. All materials potentially exposed to human material are treated with a 10% bleach solution for a minimum of 10 minutes, double bagged for autoclaving, or incinerated.
3. This kit designed for research purposes only.
4. Ensure that lab coat, gloves, and eye protection are work when working with chemicals.
5. The Buffer RL solution contains guanidinium salts which are highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

BEFORE STARTING

Label tubes (need 2 extra RNase free microcentrifuge tubes)
15ml falcon tube with 100% ethanol
Prepare **Buffer RL** – 300µL per sample and add 10µL β-mercaptoethanol / 1000µL Buffer RL
(6) – 1.8mL Buffer RL + 18µLβ-mercaptoethanol
(12) – 3.6mL Buffer RL + 36µLβ-mercaptoethanol

CELL LYSATE PREPARATION

1 Excise the tissue sample from the animal.

2 ✱

Determine the amount of tissue by weighing.

3 Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.

4 Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

5 Add **300 µl** of Buffer RL to the tissue sample and continue to grind until the sample has been homogenized.
Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

6 Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

7 Add **600 µl** of RNase-free water to the lysate. Vortex to mix.

8 Add **20 µl** of reconstituted Proteinase K to the lysate and incubate at **55 °C** for **00:15:00** Vortex the tubes occasionally during incubation.

9 Spin the lysate for **00:02:00** to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided).

10 Add **450 µl** of 96-100% ethanol (provided by user) to the lysate. Vortex to mix.

BINDING RNA TO COLUMN

11 Assemble a column with one of the provided collection tubes.

12 Apply up to **650 µl** of the lysate with the ethanol (from Step 1) onto the column and centrifuge for **14000 x g, Room temperature 00:02:00**

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at 14,000 x g (~14,000 RPM).

13 Discard the flowthrough. Reassemble the spin column with its collection tube.

14 Depending on your lysate volume, repeat Step 2b and 2c as necessary.

Note: If part of the lysate has not passed into the collection tube after step 2d and the volume is less than 200 µL, continue to step 2e without additional centrifugation.

15 Apply **400 µl** of Wash Solution A to the column and centrifuge for **14000 x g, Room temperature 00:03:00** . If entire wash volume has not passed, spin for an additional minute.

16 Discard the flowthrough and collection tube. Reassemble the spin column with a new collection tube.

ON-COLUMN DNASE TREATMENT

17 Add **15 µl** of DNase I and **100 µl** of Enzyme Incubation Buffer A to the column and centrifuge at **14000 x g, Room temperature 00:02:00** . If needed, spin for an additional minute until all of the DNase I mix has passed through the column. DO NOT DISCARD FLOWTHROUGH.

18 Pipette the flowthrough from the collection tube back onto the top of the column

19 Incubate at **Room temperature** for **00:15:00** .

COLUMN WASH

20 Apply **400 µl** of Wash Solution A to the column containing DNase I mix and centrifuge for **14000 x g, Room temperature 00:02:00** . Discard the flowthrough. Reassemble the spin column with its collection tube.

21 Repeat step 4a to wash column a second time.

22 Spin column for **00:02:00** to thoroughly dry the resin. Discard the collection tube.

RNA ELUTION

23 Place the column into a fresh 1.7 mL Elution tube provided with the kit.

24 Add **75 µl** of Elution Solution A to the column.

25 Centrifuge for **2000 rpm, Room temperature 00:02:00** , followed by **14000 rpm, Room temperature 00:02:00**

> Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat Steps 4b and 4c).

STORAGE OF RNA

26 The purified RNA sample may be stored at **-20 °C** for a few days. It is recommended that samples be placed at **-70 °C** for long term storage.

Reagents should remain stable for at least **1 year** in their unopened containers. Kits older than 1 year are not recommended for use for RNA purification for downstream NGS applications.

The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.
The RNA area should be located away from microbiological work stations
Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination

There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water

Clean all surfaces with commercially available RNase decontamination solutions
When working with purified RNA samples, ensure that they remain on ice during downstream applications