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RNA extraction from field-collected brain tissue samples from suspect rabid animals

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1 Works for me dx.doi.org/10.17504/protocols.io.bdcei2te

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ABSTRACT

This protocol details the steps involved to perform RNA extraction on rabies virus brain tissue samples collected by the WHO recommended "straw method" in the field (Meslin F-X, Kaplan MM, Koprowski H. Laboratory techniques in rabies. World Health Organization; 1996.[10]). The protocol is field-friendly and can be performed using portable, battery powered equipment.

MATERIALS

NAME	CATALOG #	VENDOR
DNA decontaminating solution (DNAaway, 10% bleach, etc.)		
1.5ml Eppendorf tubes		
Ethanol		
RNaseZap™ RNase Decontamination Solution	AM9780	Thermo Fisher Scientific
DNA/RNA Shield	R1100-50	Zymo Research
mySPIN™ 12 Mini Centrifuge	75004081	Thermo Fisher
Terralyzer	S6022	Zymo Research
Quick-RNA Miniprep Kit	R1054	Zymo Research
Wooden applicator stick / toothpicks		
Fisherbrand Reinforced 2ml tubes with screw caps a nd o-rings quantity 500 RNase/DNase free	15545809	Fisher Scientific
Ceramic Beads 1.4 mm	13113-325	Qiagen
Filter paper		

SAFETY WARNINGS

Rabies samples must be handled in containment level (CL) 2+ or CL3 conditions until sample inactivation (i.e. section 1 of the protocol). In the field or low resource settings samples can be processed to the point of inactivation in a portable glove box (e.g. UY-33666-50, Cole Parmer). Sample handlers **must** wear appropriate personal protective equipment, have received training and rabies pre-exposure prophylaxis prior to handling samples.

BEFORE STARTING

Prepare the biosafety cabinet or portable glovebox:

- Decontaminate surfaces and pipettes with UV (15mins) then wipe down with decontamination wipes or 10% bleach solution and RNAseZap
- Ensure you have a waste bag, 10% bleach filled waste pot and spray and all consumables/reagents for section 1 inside the glovebox

If samples are frozen, allow to defrost and equilibrate to room temperature

Sample preparation

1 Brain tissue samples collected in the field may be stored in glycerol-saline, RNA Later or DNA/RNA shield according to the resources available to the sample collector. Instructions to process commonly received samples for use with the **Zymo Research Quick-RNA miniprep kit** are indicated below (for other sample types please refer to the kit instruction manual)

DNase I should be included in the kit (R1054/R1055) but please confirm this is the case before beginning - we have experienced that this is not always the case for certain versions of the kit that may still be in distribution.

1.1 **Homogenised samples stored in DNA/RNA shield**

- Transfer **350 µl** of homogenised sample to a new **2 ml** screw cap tube using a pipette or disposable plastic pastette
- Add **350 µl** of RNA Lysis Buffer (1:1) and mix well

1.2 **Samples stored in RNA later/glycerol-saline**

- Prepare a homogeniser tube by adding 1.4mm ceramic beads (use a 0.2ml PCR tube to measure approx. amount of beads) to a **2 ml** reinforced tube and then add ~ **1 ml** of RNA/DNA shield using a pipette or disposable plastic pastette
- Remove a small piece of tissue* (50-100mg) from RNA later/glycerol using a wooden applicator stick/toothpick/forceps and dab excess liquid on filter paper

*If the sample has liquefied:

- Transfer **200 µl** of liquid to a new 2ml screw cap tube using a pipette or disposable plastic pastette
- Add **200 µl** of RNase-free water or PBS to the sample (1:1). Then add 4 volumes of RNA Lysis Buffer (4:1) and mix.

- Add tissue to the prepared homogeniser tube and ensure the lid is screwed on securely
- Insert tube into the lysis chamber on the Terralyzer and replace chamber shield
- Homogenise the sample for **00:02:00 approx.** and then in **00:00:30 pulses** (if required) until the sample is fully homogenised.

Notes on homogenisation:

- Tissue samples harden in RNA later, therefore may require a longer homogenisation
- If the Terralyzer gets hot, leave to cool for few minutes before using again
- It may be difficult to see if the sample is fully homogenised due to foam- leave so settle for a few minutes and homogenise again if required

- **Leave for 00:02:00 to allow sample inactivation.**
- Transfer **350 µl** of homogenised sample to a new 2ml screw cap tube
- Add **350 µl** of RNA Lysis Buffer (1:1) and mix well.

RNA extraction

2 RNA extraction and purification is performed using the **Zymo Research Quick-RNA miniprep kit.** The following steps summarise the manufacturer's instructions:

All centrifugation steps should be performed at **10000 x g** - **16000 x g** for **00:00:30** unless otherwise specified.

2.1 Transfer the sample lysed in RNA Lysis Buffer (**700 µl**) into a Spin-Away Filter column (yellow) in a collection tube and centrifuge to remove the majority of genomic DNA. **Save the flow-through.**

To process samples >700 µl, Zymo-Spin columns may be reloaded

2.2 Add a 1:1 volume of ethanol (95-100%) to the sample flow-through and mix well by pipetting up and down

2.3 Transfer the mixture to a Zymo-Spin IIICG column (green) in a collection tube and centrifuge. Discard the flow-through.

2.4 Perform an on-column DNase I treatment:

Prior to use, reconstitute the lyophilized DNase I as indicated on the vial. Store frozen aliquots.

1. Add **400 µl** RNA Wash Buffer to the column and centrifuge. Discard the flow-through.
2. In an RNase-free tube, add **5 µl** DNase I to **75 µl** DNA Digestion Buffer* and mix. Add the mix directly to the column matrix (try not to touch the filter matrix with the pipette tip).
3. Incubate the column at room temperature for **00:15:00**

*If preparing multiple samples make a mastermix

2.5 Add **400 µl** RNA Prep Buffer to the column and centrifuge. Discard the flow- through.

2.6 Add **700 µl** RNA Wash Buffer to the column and centrifuge. Discard the flow-through.

2.7 Add **400 µl** RNA Wash Buffer and centrifuge the column for **00:02:00** to ensure complete removal of the wash buffer. Transfer the column carefully into a **1.5 ml** eppendorf tube (you can discard the collection tube).

2.8 Add **50 µl** DNase/RNase-Free Water directly to the column matrix and centrifuge. **Keep the flow-through: this is the purified RNA!**

The eluted RNA can be used immediately or stored at ≤ **-70 °C** .