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

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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.

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Preparation of biosafety cabinet or portable glovebox:
- Decontaminate surfaces and pipettes with UV (15 mins) 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

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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.