RNA extraction of brain stem tissue for post-mortem diagnosis of rabies in animals by the LN34 real-time RT-PCR assay

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Abstract

Purpose

To describe the procedure of RNA extraction on post mortem brain tissue specimen using the Direct-zol™ RNA MiniPrep kit (Zymo). The extracted RNA is used in the diagnosis of lyssavirus infection by the LN34 pan-lyssavirus real-time RT-PCR assay.

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Guidelines

Purpose

To describe the procedure of RNA extraction on post mortem brain tissue specimen using the Direct-zol™ RNA MiniPrep kit (Zymo). The extracted RNA is used in the diagnosis of lyssavirus infection by the LN34 pan-lyssavirus real-time RT-PCR assay.

Responsibility

Technician will perform the procedure according to the protocol. Once the RNA extraction procedure is complete, the technician is to clean the work area, discard any biohazardous waste, and return the samples to storage.

Specimens

Homogenized brain tissue representing a full cross section of brain stem in TRIzol reagent

Before start

Definitions and Keywords

BSC – Biological Safety Cabinet

PPE – Personal Protective Equipment

RT-PCR – Reverse Transcription-Polymerase Chain Reaction

RNA – Ribonucleic Acid

RNase – Ribonuclease

Room Temperature – Temperature range of 20°C to 25°C (68°F to 77°F)

Equipment, Materials and Reagents

1. Supplies
    1. Accessioning labels
    2. Disposable gloves
    3. Sterile polypropylene micro-centrifuge tubes (1.5 and 2 ml, RNase/DNase free)
    4. Aerosol (filter) barrier tips (RNase/DNase free)
    5. Rack for Microcentrifuge tubes
    6. Ice
    7. MagNA Lyser green beads (Roche) (optional)
2. Equipment and Instruments
    1. BSC (Class II)
    2. Pipettes
    3. Vortex
    4. Microcentrifuge
    5. Freezer
    6. Mini beadbeater (optional)
3. Reagents
    1. Direct-zol™ RNA MiniPrep kit (Zymo Research)
    2. Ethanol (95% – 100%, molecular grade recommended)
4. Acceptable surface decontaminants
    1. RNase Away® (Fisher Scientific)
    2. 70% Ethanol
5. Reagent Preparation

- Add ethanol (95-100%) to Direct-zol RNA PreWash concentrate and RNA Wash Buffer concentrate as advised by the manufacturer.

Quality Control and Corrective Action

- All issues, comments, concerns, or deviations from the protocol are to be documented and brought to the attention of the lab supervisor or technical supervisor immediately if they are thought to affect the quality of the end product in any aspect.
- Temperature levels are to be monitored on applicable equipment to ensure incubators, refrigerators, and freezers stay within criteria set for acceptable temperature range.
- Specimen for RNA extraction will be assigned based on the test ordered and/or the recommendations of the technical supervisor or lab supervisor.
- All samples are to have unique identifiers and corresponding accession labels provided in advance of starting the RNA extraction procedure.

Materials

Direct-zol™ RNA MiniPrep kit R2070 - R2073 by Zymo Research

✓ Ethanol (95 - 100%), molecular grade by Contributed by users

Protocol

Set up your work space under the BSC

Step 1.

- Frozen samples should be thawed just prior to testing.
- Clean the BSC work surface using RNase Away® and 70% ethanol prior to starting work.
- Layout plastic-lined absorbent work pad(s) in the hood to perform your work on.
- Place reagents, supplies, and sample in the BSC.

Sample Preparation

Step 2.

Thoroughly homogenize brain pieces using a bead beater, micro tissue grinder or sterile applicator stick.

IMPORTANT: Ensure tissue is thoroughly homogenized. Incomplete homogenization will decrease RNA yield.

Sample Preparation

Step 3.

Let sit for at least 5 minutes at room temperature.

Sample Preparation

Step 4.

Centrifuge sample at 10,000–16,000 × *g* for 2 minutes

Sample Preparation

Step 5.

Transfer 300 µl of the supernatant into a new sterile microcentrifuge tube. Store the remaining homogenate in a screw-top tube at -20°C. The supernatant should be clear, pink liquid similar to TRIzol reagent. Avoid collection of lipids and solid tissue.

NOTE: For very little tissue (such as from a small bat), deteriorated tissue, or non-brain tissue, use 600 µl of the supernatant for extraction.

RNA Purification

Step 6.

All centrifugation steps should be performed at 10,000 – 16,000 × *g*

RNA Purification

Step 7.

Add equal volume ethanol (95–100%) to the homogenate supernatant (e.g. 300 µl ethanol to 300 µl TRIzol-supernatant) and mix thoroughly.

RNA Purification

Step 8.

Transfer 600 µL of the ethanol-supernatant mixture to a Zymo-Spin™ IIC Column in a collection tube and centrifuge until the liquid has passed through the column (1 minute).

Discard the flow-through.

Repeat if there is more than 600 µL of ethanol-Trizol mixture for a sample.

RNA Purification

Step 9.

Transfer column to a new collection tube.

RNA Purification

Step 10.

Add 400 µl Direct-zol RNA PreWash to the column and centrifuge for 30 seconds.

RNA Purification

Step 11.

Discard the flow-through, return the column to the collection tube, and repeat the 400 µL Direct-zol PreWash step.

RNA Purification

Step 12.
Discard the flow-through and return the column to the collection tube.

RNA Purification

Step 13.
Add 700 µl RNA Wash Buffer to the column and centrifuge for 2 minutes. Ensure that the wash buffer has been completely removed. Discard the flow-through and the collection tube.

RNA Purification

Step 14.
Transfer the column carefully into an RNase-free tube.

RNA Purification

Step 15.
To elute RNA, add 50 µl of DNase/RNase-Free water directly to the column matrix.

RNA Purification

Step 16.
Incubate for 30 seconds at room temperature then centrifuge for 1 minute.

RNA Purification

Step 17.
RNA is now ready for immediate use or storage. Keep extracted RNA on ice while in use. Carefully transfer RNA to new screw top flat bottom accession labeled microcentrifuge tube for long term storage at -70°C or colder.

Storage

Step 18.

- Preferably, the RNA sample should be used directly after extraction. The sample may be placed in the refrigerator for short term storage.
- If a sample will not be used for a few days, it should be stored under long term storage conditions at -70°C or colder.
- Storage at warmer temperatures or repeated freeze-thaws can lead to RNA degradation and affect diagnostic results.

Warnings

Hazards and Safety Precautions

- Wear appropriate personal protective equipment (gloves, safety glasses, and lab coat).
- Follow procedures as demonstrated in the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition: (https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf)
- Samples may contain infectious agent(s). You should be aware of the health hazard presented by such agents and should use, store, and dispose of such samples in accordance with the required safety regulations.
- Pre-exposure rabies vaccination, regular serologic tests, and booster immunizations as necessary are required for all persons prior to working with lyssaviruses or with known or potentially infected specimen, or engaging in diagnostic, production, or research activities with

these viruses (CDC, MMWR, 48: 1-22, 1999, WHO, OIE).

- Some chemicals used with this assay may be hazardous or become hazardous; refer to the SDS as needed. Dispose of chemical waste as directed in the SDS and according to local regulations.
- TRIzol reagent is a hazardous chemical; contact with acids or bleach liberates toxic gases; ensure adequate ventilation; please refer to the safety data sheet for more information.