Oct 30, 2024

RNA Extraction from Frozen Brain Tissue Using a Bullet Blender

DOI
dx.doi.org/10.17504/protocols.io.q26g7p7yqgwz/v1

Ashley V Kumar[1], Francesca Telese[1]

[1]Department of Psychiatry, University of California, San Diego

Telese Lab

Ashley V Kumar
Division of Biology, University of California, San Diego

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q26g7p7yqgwz/v1

Protocol Citation: Ashley V Kumar, Francesca Telese 2024. RNA Extraction from Frozen Brain Tissue Using a Bullet Blender. **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g7p7yqgwz/v1**

Protocol status: Working
We use this protocol and it's working

Created: January 02, 2024

Last Modified: October 30, 2024

Protocol Integer ID: 92879

Keywords: bullet blender, RNA, tissue, Zirconium Beads, RNA extraction, Trizol

Abstract

This protocol details a streamlined method for extracting high-quality RNA from small tissue samples using a bullet blender. By following this approach, researchers can efficiently isolate RNA suitable for a variety of downstream applications.

Guidelines

Temperature Control: To maintain RNA integrity, keep all steps on ice whenever possible, unless otherwise specified in the protocol.
RNase Management for RNase-Rich Tissues: For tissues high in RNases (e.g., spleen, pancreas), pre-chill TRIzol™ Reagent and other solutions to minimize RNA degradation.
Disposable, RNase-Free Equipment: Use only disposable, RNase-free pipette tips and tubes at each step to prevent contamination.
Glove Protocol: Always wear disposable gloves when handling RNA samples and reagents. Change gloves frequently—especially when transitioning from handling crude extracts to more purified materials—to minimize RNase transfer.
Workspace and Equipment Decontamination: Before starting, clean work surfaces and non-disposable items, such as centrifuges and pipettes, with RNaseZap™ RNase Decontamination Solution (Cat. no. AM9780) to ensure an RNase-free environment.

Materials

Reagents:
-RNaseZap™ RNase Decontamination Solution (Thermo Fisher, AM9780)
-TRIzol™ Reagent (Thermo Fisher, 15596026)
-Zirconium Beads (RNase Free) ZrOB05-RNA 0.5mm RNAse-free (Thermo Fisher, NC0284031)
-Eppendorf LoBind 1.5mL tubes (Eppendorf, 022431021)
-10% Tween 20 (Biorad, 1662404)
-Tris Hydrochloride, pH: 7.5 +/-0.1 (Thermo Fisher, MT46030CM)
-EDTA 0.5M pH8.0 (Thermo Fisher, 50983251)
-UltraPure DNase/RNase-Free Distilled Water (Thermo Fisher, 10977-023)
-GlycoBlue Coprecipitant (15 mg/mL) (Thermo Fisher, AM9516)
-Sodium Acetate (3 M), pH 5.5, RNase-free (Thermo Fisher, AM9740)
-Isopropanol (2-Propanol) (Sigma-Aldrich, I9516)
-Ethanol (Ethyl Alcohol) 100% - 200 Proof (DECON LABS, V1016)

Buffer:
TET [0.05% Tween, 1 mM EDTA, 10 mM Tris pH 7.5].

Equipement:
Centrifuge: (Eppendorf, Centrifuge 5430R)
Bullet Blender 24 (Next Advance, BBX24B)with Noise Reduction Enclosure (Next Advance, 4116-BBY24M, NRE-02)

Safety warnings

Centrifugation Safety: Ensure that all tubes are balanced and securely capped during centrifugation to prevent leakage or loss of sample. Follow the manufacturer's guidelines for the maximum speed.
TRIzol™ Reagent Hazard: TRIzol™ and similar reagents contain phenol and guanidine isothiocyanate, which are toxic and can cause chemical burns. Always handle TRIzol™ in a fume hood and avoid inhaling fumes or direct skin contact.

Before start

- Bring centrifuges down to 4°C and follow guidelines for **Workspace and Equipment Decontamination**

Tissue homogenization and RNA extraction

1 Begin by resuspending the tissue sample in 500 µL of chilled TRIzol™ Reagent. Transfer the mixture to a Safe-Lock tube, ensuring that the sample remains cold throughout handling. Add 10 µL of RNase-free Zirconium Beads (0.5 mm; Cat. no. ZrOB05-RNA) to the tube. Perform all steps on ice.

2 Place the prepared Safe-Lock tubes in the Bullet Blender and process at speed 6 for 1 minute in a cold room. To optimize RNA quality, keep blending time as short as possible. Inspect samples after blending—if any tissue remains unhomogenized, repeat the processing step only for those specific samples, taking care not to over-homogenize.

3 Briefly centrifuge the tubes at 1,000 rpm for 30 seconds at 4°C to bring any remaining TRIzol™ Reagent down from the tube walls and lid for optimal RNA extraction. Carefully transfer the supernatant to a new RNase-free tube kept on ice

4 Add 500 µL of chilled TRIzol™ Reagent to the new tube containing the supernatant, bringing the final volume to 1 mL. Mix gently to ensure the sample is fully suspended in TRIzol™.

5 Working under the fume hood, add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis. Immediately cap the tube securely to prevent evaporation. If using TRIzol™ LS, add 0.2 mL of TET buffer (0.05% Tween, 1 mM EDTA, 10 mM Tris, pH 7.5) instead of chloroform.

6 Incubate for 2–3 minutes at room temperature to facilitate phase separation.

7 Briefly vortex the tube for 3–5 seconds, just until the mixture appears homogeneous. Avoid over-vortexing, as this may impact RNA integrity.

8 Centrifuge the sample at 12,000 × g for 10 minutes at 4°C using a swinging bucket rotor. This centrifugation step will result in the separation of the mixture into three layers: a lower red phenol-chloroform phase (proteins and lipids), an interphase (DNA), and an upper colorless aqueous phase (RNA).

9 While the centrifugation is in progress, add 1.5 µL of RNase-free GlycoBlue™ Coprecipitant to a new, properly labeled tube. Note that GlycoBlue™ co-precipitates with the RNA, aiding in visualization without interfering with downstream applications.

10 Working under the fume hood, carefully transfer the upper, RNA-containing aqueous phase to the prepared tube (with GlycoBlue™) at room temperature. Tilt the tube at a 45° angle and use a pipette to gently draw out the aqueous layer, being careful not to disturb or transfer any of the underlying TRIzol™ or interphase. If there is any risk of contamination from the lower layers, perform a quick re-spin to ensure complete separation before attempting the transfer again.

Precipitation, Washing and Elution

11 On the bench, add 1/10th volume of 3M sodium acetate (NaOAc) to the RNA-containing aqueous phase. Mix thoroughly by briefly vortexing, then spin down the tube to collect the contents at the bottom.

12 Next, add 1 volume of 100% isopropanol to the aqueous phase mixture. Then, vortex well to ensure thorough mixing

13 Allow the RNA to precipitate by incubating the mixture for 20 minutes on ice, or alternatively, overnight at -20°C for maximum yield. **Note:** Avoid precipitation at -80°C, as any residual TRIzol ™ may also precipitate at this temperature, potentially contaminating the RNA.

14 Centrifuge the tube at 28,000 × g (maximum speed) for 30 minutes at 4°C using a fixed-angle rotor. This step will pellet the RNA at the bottom of the tube for subsequent washes.

15 Discard the supernatant with a micropipette, taking care not to disturb the RNA pellet. Quick spin and remove any leftover liquid with a micropipette.

16 Add 1 mL of 75% ethanol per 1 mL of TRIzol™ Reagent. Gently invert the tube to ensure the ethanol fully contacts the pellet and moves it around the tube. If needed, the RNA can be stored in 75% ethanol up to 1 year at -20°C or at least 1 week at 4°C.

17 Centrifuge the tube at 500 × g for 1–2 minutes at 4°C (a quick spin is also acceptable) to bring the RNA pellet to the bottom. remove and discard the supernatant using a micropipette. Quick spin, then use a P20 pipette to carefully remove any remaining ethanol at room temperature.

18 Let the RNA pellet air dry at room temperature for 5 minutes, or until it loses its shiny appearance.

19 Add 12 µL of RNase-free water or TET to the pellet, adjusting the volume as needed for your desired concentration. Incubate at 55–60°C for 2 minutes, then immediately place on ice. Proceed to downstream applications or store at –80°C.