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Brain Tissue RNA Extraction -- University of Minnesota TMCs

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Cellular Senescence Net… UMN SenNet

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We use this protocol and it's working

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Abstract

RNA extraction protocol from snap frozen tissues and/or cell pellets adapted from PureLink RNA Mini Kit and PureLink with TRIzol Reagent protocols (attached). Protocol here used with neurological tissues prior to Bulk RNA sequencing.

SOPs_RNA Extraction Neuro (1).pdf 506KB

PureLink_RNA_Mini_Kit Protocol.pdf 547KB

Purelink with Trizol.pdf 135KB

Guidelines

See Appendix in attached PDF - SOPs_RNA Extraction Neuro (1).pdf 506KB

Materials

70% ethanol spray
RNase away
two containers
dry ice
cutting board
forceps
razor

TRIzolTM Reagent
fume hood
wet ice
cold room
tissue homogenizer
chloroform
cold room centrifuge
70% ethanol (column-based method)
isopropanol (column-free method)

wash buffer I
wash buffer II
room temperature centrifuge
RNase-free water
vacuum cetrifuge
heat block

Preparation

1 Choose and locate samples: box year and number, slot number.

1.1 Note sample information (e.g., specimen ID, animal ID, genotype, age, sex, etc).

1.2 Tip: Note the quantity of tissue for each sample. If low, cutting tissue is not needed.

2 Spray benchtop with 70% ethanol or RNase away.

3 Fill two containers with dry ice. One for the tools in step #4. Second for holding tissue samples.

4 Wipe cutting board, razor, and forceps all with 70% ethanol.

> **Note**
>
> Tip: RNase away can freeze, so avoid spraying it on anything touching dry ice.

> **Note**
>
> Discard the razor into a sharps container.

5 Set (per sample) one lysing tube (i.e., green capped and contains tiny white beads), three 1.5 ml tubes, one spin cartridge with a collection tube, and one collection tube aside. Label.

6 DNase Preparation (if needed): Resuspend DNase with 550 µl RNase-free water (provided).

6.1 Create a master mix. Each sample needs 80 µl
- i. 10X DNase I reaction buffer: 8.0 µl
- ii. Resuspended DNase: 10.0 µl
- iii. RNase-free water: 62.0 µl

Homogenize Snap Frozen Tissues

7 Grab TRIzolTM Reagent from the 4°C fridge Place in a fume hood.

8 Pipette 1000 µl TRIzolTM into each lysing tube in the fume hood. [1]

9 Cut tissue from each sample (if applicable) and put in the lysing tube. Invert to mix.

9.1 Ensure all tissue is immersed in TRIzol. Each sample has its own lysing tube.

9.2 Wipe all tools with 70% ethanol before, between, and after touching samples. c.

9.3 **Tip:** Store samples in TRIzolTM at -80°C before homogenizing, if you wish to stop at this step.

10 Replace the dry ice in the first container with wet ice (**optional**–only if you wish to cool lysing tubes on wet ice instead of the cold room).

11 Homogenize all samples in tissue homogenizer. [2]

11.1 a. Select tissue type within recommended programs. Most run for 40 seconds. [3]

11.2 b. Repeat homogenization until tissue appears a fine powder within the TRIzol liquid.
i. Tip: The smaller the tissue, the less homogenization needed. For brain regions, one typically will only need one round of 40 second homogenization. For larger organs (e.g., liver, kidney, spleen) or greater tissue quantity, three rounds may be necessary.
ii. **Tip**: Pink foam is commonly seen after homogenizing brain regions. Foam will disappear in a few minutes. Occurs due to the speed the tubes are spinning.

12 If homogenizing more than once, place all lysing tubes on wet ice OR place in a cold room for 2-mins between homogenization runs. Cooling is necessary to prevent RNA degradation from heat produces while the samples were being homogenized.

13 Incubate the lysate with TRIzolTM at for 5 mins.

Homogenize Cell Pellets

14 Grab TRIzolTM Reagent from the 4°C fridge Place in a fume hood.

14.1 **Tip:** Remove cell pellets from -80°C storage just before beginning the protocol. Do not let thaw before adding TRIzolTM. You may take out samples in batches of 4-6 samples.

15 Pipette 1000 µl TRIzolTM per 1 million cells into each 1.5 ml tube in the fume hood. [1]

16 Incubate in TRIzolTM for 2-3 mins.

17 Resuspend cell pellets by pipetting up-and-down 15-25 times. Keep consistent between cells.

18 Allow the tubes to sit for 5 minutes.

19 **Repeat steps #4-5** for each tube until pellets are dissolved. a. Tip: Store samples in TRIzol at -80°C before homogenizing, if you wish to stop for now.

Isolate Snap Frozen Tissue or Cell P

20 In the fume hood, add 200 µl chloroform into a new and separate 1.5 ml tube.

21 Add 1000 µl lysate with TRIzolTM into each chloroform-containing 1.5 ml tube. This is best done in the fume hood.

Note

Tip: Do not pipette the 200 µl chloroform into the lysing tube. This saves the step of re-pipetting the mixture out of the lysing tube into a new 1.5 ml tube, as beads will not allow the mixture to separate into layers.

22 Gently invert tubes for 15-30 secs. Mix thoroughly to ensure proper phase separation.

Note

Tip: You may invert tubes individually by hand or by stacking another tube rack on top and mixing all tubes at once.

23 Incubate at room temp for 2-3 mins.

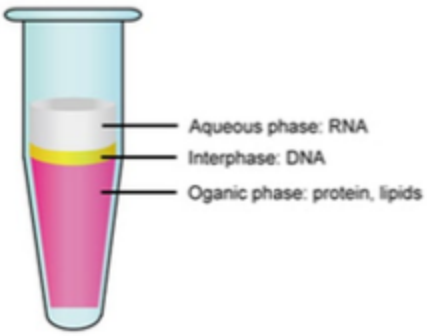
24 Centrifuge in a cold room at 4°C and 12,000xg for 15 mins.

25 Transfer 400 µl upper phase into each new 1.5 ml tube.

Note

Tip: Do two rounds of 200 µl with a P200.

25.1 **Layers:** (1) colorless upper aqueous phase containing RNA; (2) white interphase containing DNA; (3) red phenol-chloroform lower organic phase containing protein and lipids. heat produced while the samples were being homogenized.



Note

Tips: If you have lower tissue or cell quantities, layer 2 may not be visible. You can add 500 µl (tissues) or 600 µl (cells) upper phase to ensure greater RNA concentration.

26 **Column-Based Method:** Add 400-600 µl 70% ethanol into each RNA-containing tube.

26.1 Gently pipette up-and-down 4-5 times to mix. RNA and ethanol should be 1:1 ratio.

Note

Tip: Continue mixing until RNA is no longer visible in the tube.

Note

Tip: 70% ethanol aliquot can be kept at the bench at room temp in 15 ml tube.

Note

Tip: 70% ethanol = 70 ml absolute (100%) ethanol + 30 ml RNase-free water. Keep the stock solution in a 4°C fridge.

27 **Column-Free Method:** Add 0.5 ml of isopropanol to the aqueous phase to precipitate RNA, per 1 ml of TRIzolTM Reagent used for lysis.

Bind and Wash Snap Frozen Tissues or Cell Pellets (Column-Based Method)

28 Transfer 500 µl of sample into a spin cartridge with a collection tube.

29 Centrifuge at room temp and 12,000xg for 20 secs.

30 **Discard flow-through ONLY.**

31 Repeat steps #1-3 until all samples are processed.

> Note
>
> Tip: RNA will stick in the spin cartridge filter and will be eluted with RNase-free water.

32 Add 700 µl wash buffer I to the spin cartridge.

33 Centrifuge at room temp and 12,000xg for 20 secs

34 **Discard flow-through AND collection tube.** Insert spin cartridge into new collection tube.

34.1 **Note: *steps #5-6*** (i.e., wash buffer I steps) differ if DNase is to be added.
i. Add 350 µl wash buffer I to the spin cartridge.
ii. Centrifuge at room temp and 12,000g for 20 secs.
iii. **Discard flow-through AND collection tube.** Insert spin cartridge into new collection tube.
iv. Add 80 µl PureLink DNase mixture to the center of the spin cartridge.
v. Incubate at room temp for 15 mins.
vi. Repeat steps #7 i-iii once more. Proceed to ***step #8***.

35 Add 500 µl wash buffer II to the spin cartridge and new collection tube.

36 Centrifuge at room temp and 12,000xg for 20 secs.

37 **Discard flow-through ONLY.**

38 Repeat steps #8-10 once more.

39 Centrifuge at room temp and 12,000xg for 1 min to dry the spin cartridge before elution.

40 **Discard flow-through AND collection tube.** Insert spin cartridge into new 1.5 ml tube.

ELUTE SNAP FROZEN TISSUES (Column-Based Method)

41 Add 30 µl RNase-free water to the center of the spin cartridge.

42 Incubate at room temp for 30 secs.

43 Centrifuge at room temp and 12,000xg for 2 mins. Transfer eluent to a new 1.5 ml tube if broken.

> **Note**
>
> If you added DNase, reduce spin time to 1 min.

44 Store at -80°C. Avoid leaving RNA at room temp – Keep on wet ice.

Elute Cell Pellets (Column-Based Method)

45 Add 20 µl RNase-free water to the center of the spin cartridge.

46 Incubate at room temp for 30 secs.

47 Centrifuge at room temp and 12,000xg for 2 mins.

48 **Important:** Insert spin cartridge into new 1.5 ml tube. Keep the eluent for ***step #5***.

49 Add the eluent (i.e., 20 µl RNase-free water from ***step #1***) to the center of the spin cartridge.

50 Incubate at room temp for 30 secs.

51 Centrifuge at room temp and 12,000xg for 2 mins.

52 Add new 10 µl RNase-free water to the center of the spin cartridge.

53 Incubate at room temp for 30 secs.

54 Centrifuge at room temp and 12,000xg for 2 mins. Transfer eluent to a new 1.5 ml tube if broken.

55 Store at -80°C. Avoid leaving RNA at room temp – Keep on wet ice.

Wash and Solubilize Cell Pellets (Column-Free Method)

56 Wash the RNA

56.1 Resuspend the cell pellet in 1 ml of 75% ethanol per 1 ml of TRIzol™ Reagent used for lysis.

56.2 Mix the sample briefly.

56.3 Centrifuge in a cold room at 4°C and 7,500xg for 5 mins.

> **Note**
>
> The RNA can be stored in 75% ethanol for at least 1 year at –20°C, or at least 1 week at 4°C.

56.4 Discard the supernatant with a micropipette.

56.5 Air dry the RNA pellet for ~ 30 minutes at room temp.

> **Note**
>
> To expedite drying, put tubes in vacuum centrifuge (i.e., lyophilizer) for 1-2 minutes. The purpose of drying isn't to remove water, but rather to evaporate any remaining ethanol. Ethanol is much more volatile than water and evaporates more quickly, especially under vacuum.

57 Solubilize the RNA

57.1 Resuspend the RNA pellet in 20–50 µl RNase-free water by pipetting up-and-down.

57.2 Incubate in a heat block set at 55–60°C for 10–15 minutes.

57.3 Proceed to downstream applications, or store the RNA at –70°C.