

Purification of RNA from *C. elegans* using TRIzol[®] Reagent (Life technologies #15596) and Direct-zol[™] RNA MiniPrep columns (Zymo Research #R2050)

Overview

Obtaining high-quality RNA is the first, and often the most critical, step in performing many molecular techniques such as reverse transcription PCR (RT-PCR), transcriptome analysis using next-generation sequencing, array analysis, northern analysis, and cDNA library construction. This procedure is complicated by the ubiquitous presence of ribonuclease enzymes in cells and tissues, which can rapidly degrade RNA. To counter this, equipment used for RNA extraction is usually cleaned thoroughly, kept separate from common lab equipment and treated with various harsh chemicals that destroy RNases. For the same reason, it is important to take special care not to let bare skin touch the equipment.

Several methods are used in molecular biology to isolate RNA from samples. We will be using TRIzol[®], a ready-to-use reagent designed to isolate high quality total RNA from cell and tissue samples from human, animal, plant, yeast, or bacterial origin. TRIzol[®] Reagent maintains the integrity of RNA due to highly effective inhibition of RNase activity while disturbing the cells and dissolving cell components during sample homogenization. Following tissue homogenization, the RNA will be purified using the Direct-zol[™] RNA MiniPrep columns. These columns provide a streamlined method for the purification of up to 50 µg (per prep) of high-quality RNA directly from samples in TRIzol[®] Reagent.

Important points before starting

- **Caution! TRIzol[®] Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Always work with TRIzol[®] in a fume hood, and always wear a lab coat, gloves and safety glasses.**
- Ensure that RNA isolation is performed in an RNase-free environment. Wipe the bench surface and pipettes with RNase Away solution.
- Perform all steps of the procedure at room temperature. All centrifugation steps should be performed at maximum speed (14,000 rpm) unless otherwise noted.
- All solutions (except the final eluate with water) are hazardous waste and must be collected and then added to the designated container in the Satellite Accumulation Area (SAA) in the chemical fume hood.

Procedure

- 1) Wash worms off plates with 1 ml M9 buffer and collect them in 1.5 ml centrifuge tubes.
- 2) Spin at 2,000 rpm for 1 minute to pellet the worms. Carefully remove the supernatant, leaving a small volume intact to avoid disturbing the worm pellet.
- 3) Wash 2X with M9 buffer to remove bacterial carry over. Resuspend worm pellet with 1

ml M9 Buffer, spin at 2,000 rpm for 1 minute and remove supernatant. Repeat twice.

- 4) Add 1 ml TRIzol[®] for 100 μ l worms. Adjust TRIzol[®] volume as necessary, depending on the size of your worm pellet.
Note: at this step, you can freeze tubes and store at -80 °C until you are ready to proceed.
- 5) Vortex worms/ TRIzol[®] mixture for 1 minute.
- 6) Let stand at room temperature for 10 minutes, vortexing occasionally.
- 7) Add one volume ethanol (95-100%) directly to one volume sample homogenate (1:1) in TRIzol[®] reagent. Mix well by vortexing.
- 8) Load the mixture into a **Zymo-Spin II Column** in a collection tube and centrifuge for 1 minute at maximum speed (14,000 rpm). Transfer the column into a new collection tube. (Note: discard the flow-through in the appropriate waste container in the SAA).
- 9) Wash the column with 400 μ l **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.
- 10) For each sample, prepare **DNase I Reaction Mix** in an RNase-free tube. Add the reagents in the following order:

		<u>For 3 samples</u>
a. DNase I	5 μ l (1U/ μ l)	15 μ l
b. 10X DNase I Reaction Buffer	8 μ l	24 μ l
c. DNase/RNase-Free Water	3 μ l	9 μ l
d. RNA Wash Buffer	<u>64 μl</u>	192 μ l
	80 μ l total/sample	
- 11) Add 80 μ l of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature for 15 minutes, then centrifuge for 30 seconds. Discard flow-through.
- 12) Add 400 μ l **Direct-zol RNA PreWash** to the column and centrifuge for 1 minute. Discard the flow-through. Repeat this step.
- 13) Add 700 μ l **RNA Wash Buffer** to the column and centrifuge for 1 minute. Discard the flow-through. To ensure complete removal of the wash buffer, centrifuge the column for an additional 2 minutes.
- 14) Transfer the column carefully into an RNase-free tube.
- 15) Add 25 μ l of **DNase/RNase-Free water** directly to the column matrix, let it stand for 1 minute, followed by centrifugation for 1 minute. Note: it is crucial that RNA is eluted and stored in safe, RNase-free water.
- 16) The eluted RNA can be used immediately or stored at -70C. Note: RNA is a highly unstable molecule. After elution, the purified RNA should be kept in ice at all times to prevent degradation.

Determining RNA purity using the NanoDrop

260/280 Ratio

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

260/230 Ratio

This ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants that absorb at 230 nm.

References

Adapted from Direct-zol RNA MiniPrep protocol, Zymo Research. <http://www.zymoresearch.com/rna/total-rna-purification/samples-in-tri-reagent-trizol-etc/direct-zol-rna-miniprep>.

Adapted from Trizol Reagent protocol, Life technologies. https://tools.lifetechnologies.com/content/sfs/manuals/trizol_reagent.pdf

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