RNA extraction using the 'home-made' Trizol substitute

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Abstract

A simple protocol for RNA extraction from various types of samples (protists, fungi, bacteria, organelles, subcellular fractions, ribosomes, etc.). Compared to the commercial Trizol® procedure, it makes use of generally available chemicals without the need for columns, thus allowing concurrent extraction of transcripts of all sizes (including RNAs <200 nt). Small circular DNA molecules (up to ~9 kbp) will also be efficiently isolated. The procedure is based on a previously published protocol by Rodríguez-Ezpeleta *et al*. (DOI: 10.1007/978-1-60327-136-3_3).

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Guidelines

- Stored in a glass bottle protected from light (e.g. wrapped in aluminium foil) and kept at 4 °C, the home-made Trizol substitute solution is stable for at least 3 months. Discard if pink or purple coloration indicative of phenol oxidation appears.
- The procedure is scalable. Its yield, as tested with spiked-in radioactively labeled transcripts of 60-900 nt, is 70-90% (in general, increasing with size). The minimal amount of RNA that could be reproducibly extracted was 10 ng (at a concentration of 200 pg/µl).
- Note that RNA extracted using this protocol is usually not DNA-free, so DNase treatment might be necessary. Circular DNA molecules up to ~9 kbp are isolated at high yields.

Materials

- Isopropanol by Contributed by users
- Glycerol G5516 by Contributed by users

  Phenol, Saturated, pH 4.3, Liquid BP1751I by Fisher Scientific

  Guanidine Thiocyanate BP221 by Fisher Scientific

  Ammonium Thiocyanate A709 by Fisher Scientific
- Sodium Acetate, Trihydrate by Contributed by users
- Chloroform by Contributed by users
- Ethanol by Contributed by users

  Glycogen AM9510 by Thermo Scientific

Protocol

Step 1.
Prepare the components for the home-made Trizol substitute:

Component	Final concentration	100 ml
Phenol, Saturated (pH 4.3, Liquid)	38 %	38 ml
Guanidine Thiocyanate	0.8 M	11.82 g
Ammonium Thiocyanate	0.4 M	7.61 g
Sodium Acetate pH 5.0 (3M solution)	0.1 M	3.33 ml
Glycerol	5 %	5 ml
Water (ddH_2O)		to 100 ml

Step 2.
Mix the components and stir at room temperature until completely dissolved (30-60 minutes). Do not heat the solution. Store at 4 °C in a glass bottle protected from light.

Step 3.
Add 5 volumes of the home-made Trizol substitute to the sample.

- *When working in 1.5 mL tubes, for practical reasons, the minimal and maximal volume of the sample is 30 and 200 µl, respectively.*

Step 4.
Vortex vigorously for 10-30 seconds (depending on the viscosity and protein and nucleic acid concentration of the sample).

Step 5.
Incubate at room temperature for 5 minutes.

Step 6.
Add 1 volume of chloroform (relative to the original volume of the sample).

Step 7.
Mix vigorously 10-20 times by inverting the tube.

- *Do not vortex to avoid breaking DNA.*

Step 8.
Incubate at room temperature for 5 minutes.

Step 9.
Spin for 10-15 minutes at >12,000 g and 4 °C (depending on the viscosity and protein and nucleic acid concentration of the sample).

Step 10.
Transfer the upper aqueous phase into a new tube.

- *Avoid the white DNA precipitate at the interphase. If the sample volume was 100 µl, the*

aqueous phase should be 450 µl.

Step 11.
Add 1.1 volumes of isopropanol (relative to the volume of the aqueous phase).

- *If low RNA content is expected in the sample, prior to isopropanol, add >10 µg of glycogen to facilitate precipitating nucleic acids and spotting the pellet. Recommended final concentration of glycogen is 50–150 µg/ml.*

Step 12.
Mix well by inverting the tube.

Step 13.
Incubate 30-60 minutes at 4 °C or at room temperature.

Step 14.
Spin for 30-45 minutes at >12,000 g and 4 °C.

Step 15.
Discard the supernatant.

Step 16.
Add 1 volume of 70% (aq.) ethanol (relative to the volume of the aqueous phase).

Step 17.
Mix gently.

- *Ensure that the pellet is submerged in the solution and not sticking to the tube wall.*

Step 18.
Incubate 10 minutes at room temperature.

Step 19.
Spin for 10 minutes at >12,000 g and 4 °C.

Step 20.
Discard the supernatant.

Step 21.
Let the pellet dry at room temperature, so that no traces of 70% ethanol remain.

- *Drying under vacuum is not recommended because of overdrying that makes it harder to dissolve the pellet.*

Step 22.
Completely dissolve the pellet in RNase-free water.

Warnings

- Phenol causes heavy skin burns and is toxic in skin contact or vapor inhalation. Manipulate under a fume hood, using gloves.
- Guanidine thiocyanate and ammonium thiocyanate are harmful by inhalation, in skin contact

and if swallowed. Their contact with acids liberates toxic gas. Manipulate under a fume hood, using gloves.
- Chloroform is harmful if swallowed, irritates eyes, respiratory system and skin.
- Isopropanol and ethanol are flammable (liquid and vapor), may cause irritation of respiratory tract, and may cause drowsiness and dizziness.