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RNA Extraction Without a Kit

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.beabjaan

Coronavirus Method Development Community

Addgene The Nonprofit Plasmid Repository
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ABSTRACT

This protocol describes RNA extraction without a kit. To see the full abstract and additional resources, visit https://www.addgene.org/protocols/kit-free-rna-extraction/

EXTERNAL LINK

https://www.addgene.org/protocols/kit-free-rna-extraction/

GUIDELINES

This protocol was adapted from Chomczynski P and Sacchi N, 2006 and TRIzol® User Guide from ThermoFisher Scientific.

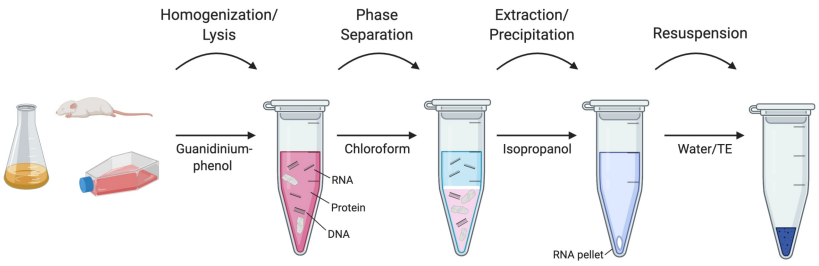

Figure 1: A diagram of the different steps in RNA extraction.

MATERIALS TEXT

Equipment

- Refrigerated microcentrifuge

if you only have a non-refrigerated microcentrifuge, see step 5 of either protocol before you start

- Homogenizer
- Vortexer
- -20C freezer
- -80C freezer

Materials/Reagents

- Solution D (for Protocol Option #1):

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- 4 M guanidinium thiocyanate,
- 25 mM sodium citrate, pH 7.0
- 0.5% (wt/vol) N-laurosylsarcosine (Sarkosyl)
- 0.1 M 2-mercaptoethanol

- TRIzol® or similar product such as TRI Reagent®, RNAzol®, QIAzol® (for Protocol Option #2)
- Water-saturated phenol
- 2 M sodium acetate pH 4
- Chloroform/isoamyl alcohol (49:1)
- 75% Ethanol
- RNase-free water or TE solution
- RNase free tubes: microcentrifuge tubes, 4 mL polypropylene tubes
- RNase decontamination solution like RNase AWAY® or RNaseZap®
- Isopropanol (for precipitation step, Option A)
- 7.5 M Lithium Chloride (for precipitation step, Option B)
- Glycogen (Optional)

Make sure to read the SDS (Safety Data Sheet) for safety warnings and hazards for these reagents. Work in a well-ventilated space and under a fume hood when working with the volatile reagents in the list above.

SAFETY WARNINGS

Make sure to read the SDS (Safety Data Sheet) for safety warnings and hazards for the reagents listed in this protocol. Make sure to work in a well-ventilated space and under a fume hood when working with the volatile reagents in the reagent list.

BEFORE STARTING

Before Starting

- RNA is not as stable as DNA and is susceptible to degradation by heat, RNases, and other enzymes.

Pro- Tips

- Always wear gloves, and whenever possible, keep RNA sample and reagents cold and work quickly to reduce RNA degradation.
- Keep work area, equipment, and reagents RNase-free (Use an RNase decontamination solution, such as RNaseZap® or RNase AWAY®, may be used).

- For more tips on working with RNA, read this blog post on RNA extraction without a kit.

Homogenization/Lysis

1 If you are using Solution D, start with step-case 'Solution D'. If you are using TRIzol®, TRI Reagent®, RNAzol®, or QIAzol®, start with step-case 'TRIzol®'

See the Materials section for recipes and reagents.

step case

Option #1 - Solution D Protocol

Before Starting this protocol, prepare a stock of solution D (see reagent section for recipe).

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2 Homogenize or lyse tissues or cells in Solution D.

- For tissues: use **1 ml** of Solution D per **100 mg** of cells.
- For cultured cells: use **1 ml** of Solution D per 1 X 10^7 cells.

3 Allow sample(s) to sit at **Room temperature** for **00:05:00** to allow for dissociation of the nucleoprotein complexes.

The effectiveness of your RNA isolation will depend on how effective your cell lysis protocol is. While simple homogenization is effective for most mammalian tissues, more hardy tissues such as bone, or bacteria/yeast/plant samples will require additional steps to effectively lyse open the cells.

Extraction

4 Extract RNA from the homogenized sample(s). Transfer tissue/cell lysate to a **4 ml** tube. Add the following sequentially to **1 ml** of lysate:

- Add **0.1 ml** of **2 Molarity (M)** sodium acetate **pH 4**, mix thoroughly by inversion.
- Add **1 ml** water-saturated phenol, mix thoroughly by inversion.

Add **0.2 ml** of chloroform/isoamyl alcohol (49:1) and then shake vigorously by hand for **00:00:10** .

5 Incubate sample(s) for **00:15:00** **On ice** and centrifuge the sample(s) at **12000 x g, 4°C 00:15:00** to separate RNA from the rest of the tissue/cell lysate.

Pro-Tip

Having your samples spin at **4 °C** helps reduce RNA degradation. If you don't have access to a refrigerated centrifuge, you can carefully bring a centrifuge into a cold room for centrifugation. Once you're done using the centrifuge, bring this equipment back to **4 °C** , as prolonged storage in the cold room may damage it.

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6 Using a pipettor, carefully transfer the top, aqueous phase to a new RNAse-free tube.

The mixture separates into a bottom organic layer, an interphase layer, and a top, aqueous layer. Take care to not disturb or collect the interphase layer with your pipette.

Pro-Tip
To collect as much of the top aqueous phase without disturbing the interphase layer, consider using a lower volume pipettor like a p200 to collect a majority of the aqueous phase. You may have to collect twice or more from the same tube, but unlike using a p1000 tip it will give you more control of where you're aiming your tip in the tube.

Precipitation and Resuspension

7 Precipitate your sample(s). You can use either Isopropanol or Lithium Chloride for this step.

Isopropanol (Option A) - Add 1 volume of Isopropanol to the extracted aqueous layer. Incubate at **-20 °C** for **01:00:00** .

Lithium Chloride (Option B) - LiCl selectively precipitates RNA versus DNA or proteins. Add the correct amount of **7.5 Molarity (M)** LiCl solution to bring the concentration of LiCl in the extracted aqueous layer to **2.5 Molarity (M)** . Incubate at **-20 °C** for **01:00:00** .

Pro-Tips
- If you anticipate your RNA yield to be small, RNase-free Glycogen may be used as a carrier to facilitate RNA precipitation. This does not affect the quality of RNA or downstream.
- To improve yield of RNA, instead of incubating at **-20 °C** for **01:00:00** , you can try incubating at **-80 °C** **Overnight** .

8 Centrifuge at **10000 x g, 4°C 00:20:00** and discard the supernatant. There should be a gel-like white pellet of total RNA in the bottom of the tube.

9 Wash the RNA by resuspending the pellet in **0.5 ml** – **1 ml** of 75% ethanol and vortex for a few seconds. Centrifuge at **10000 x g, 4°C 00:05:00** and remove the supernatant.

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10 Remove as much of the ethanol wash as possible without disturbing the pellet. Air-dry the pellet for **00:05:00** - **00:10:00** .

Critical
It is important to not let the pellet get too dry before resuspending, as this affects the solubility of the RNA.

Pro-Tip
To prevent overdrying, watch the pellet and carefully remove any residual ethanol wash and add RNase-free water or TE as soon as the entire tube is dried but while the white pellet is still visible.

11 Resuspend RNA pellet in RNase-free water or TE. Quantify and assess the quality of your RNA sample(s) using a spectrophotometer (such as a Nanodrop), agarose gel, or bioanalyzer. For more information on nucleic acid quantification, see our protocol on DNA quantification, which can be modified for RNA. Store your RNA sample(s) at **-80 °C** to prevent RNA degradation and avoid multiple freeze-thaw cycles.

Pro-Tip To avoid multiple freeze-thaw cycles of your entire RNA sample, consider making smaller aliquots of your original sample and storing those in **-80 °C** .

Homogenization/Lysis

step case

Option #2 - TRIzol® Protocol

Before Starting this protocol, make sure that you have an all-in-one acid guanidinium thiocyanate-phenol solution such as TRIzol®, TRI Reagent®, RNAzol®, or QIAzol®

2 Homogenize cells in TRIzol® or a similar product.

- For tissues: use **1 ml** of TRIzol® per **100 mg** of cells.
- For cultured cells: use **1 ml** of TRIzol® per 1 X 10^7 cells.

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3 Allow sample(s) to sit at **Room temperature** for **00:05:00** to allow for dissociation of the nucleoprotein complexes.

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Extraction

4 Extract RNA from the homogenized sample(s). Add **0.2 ml** of chloroform/isoamyl alcohol (49:1) per **1 ml** of TRIzol® used. Shake vigorously by hand for **00:00:10** .

5 Incubate sample(s) for **00:02:00** - **00:03:00** **On ice** and centrifuge the sample(s) at **12000 x g, 4°C 00:15:00** to separate RNA from the rest of the tissue/cell lysate.

Pro-Tip
Having your samples spin at **4 °C** helps reduce RNA degradation. If you don't have access to a refrigerated centrifuge, you can carefully bring a centrifuge into a cold room for centrifugation. Once you're done using the centrifuge, bring this equipment back to **4 °C** , as prolonged storage in the cold room may damage it.

6 Using a pipettor, carefully transfer the top, aqueous phase to a new RNAse-free tube.

The mixture separates into a bottom organic layer, an interphase layer, and a top, aqueous layer. If using TRIzol®, the bottom layer will be a red-pink color. Take care to not disturb or collect the interphase layer with your pipette.

Pro-Tip
To collect as much of the top aqueous phase without disturbing the interphase layer, consider using a lower volume pipettor like a p200 to collect a majority of the aqueous phase. You may have to collect twice or more from the same tube, but unlike using a p1000 tip it will give you more control of where you're aiming your tip in the tube.

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