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RNA extraction from organoids encapsulated in alginate norbornene using TRIzol Reagent

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Helen Kearney[1], Lorenzo Moroni[1], Carlos Mota[1]

[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, 6229 ER, Maastricht, the Netherlands

Helen Kearney
MERLN Institute for Technology-Inspired Regenerative Medicin...

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We use this protocol and it's working

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Abstract

This protocol describes the isolation of high-quality RNA from organoids encapsulated in alginate norbornene hydrogels. Organoids are first released from the hydrogel matrix using alginate lyase digestion, followed by homogenization in TRIzol and RNA extraction through standard organic phase separation. The expected outcome is the recovery of intact RNA with sufficient yield and purity for downstream molecular biology applications, including qPCR and sequencing. Major optimizations include removal of the hydrogel prior to RNA extraction to minimize contamination from residual matrix components, refinement of the homogenization step to improve reproducibility, and the inclusion of glycogen blue to facilitate visualization and recovery of small RNA pellets, particularly in low-yield samples.

Materials

Reagents

- Alginate lyase powder
- PBS (cell culture grade) or 0.1 M phosphate buffer (pH 6.3)
- TRIzol reagent (Thermo Fisher)
- Chloroform
- Isopropanol (molecular biology grade)
- Ethanol (molecular biology grade, ice-cold)
- Glycogen blue (optional, for low RNA yield)
- Nuclease-free water

Consumables

- 1.5 mL RNase-free Eppendorf tubes (tapered bottom recommended)
- 200 µL RNase-free pipette tips
- 0.2 µm syringe filter
- 3 mL syringes with luer lock
- 20-gauge needles
- Homogenization tubes (if using bead homogenizer)

Equipment

- Tabletop centrifuge (capable of 300 g, 7,500 g, and 15,000 g, refrigerated to 4 °C)
- Water bath (37 °C)
- Homogenizer (e.g., Precellys Evolution Touch) — optional if not using syringe method
- Heating block (capable of 60 °C)
- Spectrophotometer (e.g., NanoDrop) or fluorometer (e.g., Qubit)
- –20 °C freezer
- –80 °C freezer
- Liquid nitrogen (for snap freezing)

Troubleshooting

Safety warnings

- **Toxicity:** TRIzol contains **phenol and guanidinium isothiocyanate**, both of which are highly toxic and corrosive. Exposure can cause severe burns to skin, eyes, and respiratory tract.
- **PPE:** Always wear **lab coat, nitrile gloves, and safety goggles**. Use double gloves if handling large volumes.
- **Ventilation:** Handle TRIzol and chloroform inside a **certified chemical fume hood** to prevent inhalation of vapors.
- **Waste disposal:** Collect TRIzol, chloroform, and isopropanol/ethanol waste in **clearly labeled organic waste containers**. Do not dispose down the sink.
- **Spill response:** In case of a spill, absorb with inert material (e.g., paper towels, vermiculite) and dispose as hazardous chemical waste.

Alginate norbornene digestion

35m

1 Make 20U/mL Alginate Lyase solution

5m

1.1 Weigh 2mg Alginate Lyase powder per 1mL solution needed.

1.2 Dissolve alginate lyase powder in PBS. Invert to mix or gently vortex.

NOTE: Alginate lyase solution can also be prepared in 0.1 M phosphate buffer, pH 6.3, which is recommended for optimum activity. PBS for cell culture can also be used without negatively affecting enzyme activity.

1.3 Sterile filter the solution using a 0.2 µm pore filter and syringe. Store at 4 °C for up to 1 month. Warm in a 37°C water bath before use.

2 Transfer organoids encapsulated in alginate norbornene hydrogel into an Eppendorf tube. Calculate the total hydrogel volume by multiplying the number of organoids by the volume of gel each is encapsulated in (i.e. 10 organoids encapsulated in 20 µL alginate norbornene hydrogel (10 × 20 µL) = 200 µL total).

Note: A 1.5 mL tube with a tapered bottom is best to aid organoid collection after release from the gel.

3 Add an equal volume of 20 U/mL alginate lyase solution to the total hydrogel volume in each Eppendorf.

4 Incubate in a 37 °C water bath for 10–15 min.

15m

Note: Incubation time may need adjustment for hydrogels composed of alginate or higher concentrations of alginate norbornene.

5 Invert the tube to check digestion progress. Only cell pellet/free-spheroids/free-organoids should be visible at the bottom of the tube before proceeding beyond step 7.

6 Spin down cells/spheroids/organoids to gather them at the bottom of the Eppendorf tube: 300 g for 1 min.

Note: Centrifugation time and speed may need adjustment depending on the sample type. For organoid release from alginate norbornene, 300 g for 1 min, or even a table top centrifuge may be sufficient.

7 Remove the alginate lyase solution. If the hydrogel is not completely dissolved replace with the same volume of fresh solution and repeat steps 4–7. 15m

8 Wash organoids twice with PBS to remove residual enzyme. Spin down at same speed setting used in step 6.

9 Proceed directly to RNA extraction or snap-freeze samples in liquid nitrogen and store at –80 °C.

Homogenization in TRIzol reagent 10m

10 Retrieve samples and place on ice. Add 1 mL TRIzol to each sample.

Note: According to the manufacturer's guidelines, the recommended amount of TRIzol Reagent is 1 mL per 50–100 mg of tissue or per 1 × 10^7 cells. The exact volume should be adjusted relative to sample size to ensure complete lysis and optimal RNA recovery.

11 Homogenize samples: Two options 10m

11.1 Option 1: Needle + syringe: Using a 20-gauge needle and 3 mL syringe with luer lock, aspirate and dispense the sample 3–4 times to break the gel and lyse organoids in TRIzol.

11.2 Option 2: Bead homogenizer: Transfer samples to homogenization tubes and homogenize at 500 rpm for 15 sec on a homogenizer (e.g., Precellys Evolution Touch).

NOTE: Repeat cycle if desired but make sure the tube doesn't overheat as this can affect RNA. Place sample on ice between runs.

12 If not processing immediately, store homogenized samples: 4 °C overnight or –20 °C for up to 1 year.

RNA extraction 3h 21m

13 Incubate homogenized samples at room temperature for 5 min. During this incubation, set the centrifuge temperature to 4 °C for the next step. 5m

14 Add 200 µL chloroform to each sample (1:5 ratio relative to the TRIzol volume). Shake by hand for 30 sec, then incubate at room temperature for 5 min. 5m

15 Centrifuge at 15,000 g for 15 min at 4 °C. 15m

16 While samples are spinning, prepare fresh RNase-free Eppendorf tubes for collection of the aqueous phase.

17 Add 1 µL glycogen blue to each fresh tube.

Note: Glycogen Blue aids RNA pellet formation; the blue color helps visualize the pellet after isopropanol/ethanol washes. Highly recommended for low RNA yields (≈100–200 ng total).

18 Carefully remove tubes from the centrifuge without disturbing phase separation. Using a 200 µL pipette, transfer the clear upper aqueous phase (RNA-containing) into the prepared RNase-free tubes with Glycogen Blue.

Note: TRIzol + chloroform creates three phases: upper aqueous (RNA), middle interphase, and lower organic (DNA & proteins, fed). Keep the interphase + organic phase if DNA/protein isolation is needed (see TRIzol user guide).

19 Add 500 µL isopropanol (molecular grade) to the aqueous extract (1:2 ratio relative to the TRIzol volume). Shake by hand for 30 sec and incubate at –20 °C for 1–4 h. 2h

Note: Cold incubation improves RNA precipitation, especially for low yields. Do not exceed 4 h to avoid co-precipitation of salts/contaminants that depress the A260/A230 ratio.

20 Centrifuge at 15,000 g for 20–25 min at 4°C. 25m

21 Carefully remove all supernatant without disturbing the RNA pellet.

NOTE: The pellet will be a light blue colour if Glycogen Blue was used.

22 Add 600 µL ice-cold 75% ethanol (molecular grade) to wash the RNA pellet. Shake by hand 10–15 sec.

23 Centrifuge at 7,500 g for 8 min at 4 °C. 8m

24 Repeat steps 21–23 8m

Note: A second ethanol wash will give you a purer RNA product

25 Remove all liquid and air-dry the pellet for 10–15 min. 15m

Note: Remove as much ethanol as possible, including droplets from the Eppendorf tube edges. Be careful not to over-dry the RNA pellet as it can make it difficult to dissolve in the next step.

26 Add 20 µL nuclease-free water and ensure the RNA pellet dissolves. Once fully dissolved keep the RNA product chilled on ice.

Note: If RNA does not dissolve, incubate on a heat block at 60 °C for 10 min.

27 Measure RNA yield using a Spectrometer or Fluorometer.

Note: A spectrophotometer should be used to assess purity (A260/A280 and A260/A230 ratios), whereas a fluorometer can be used to provide more accurate RNA quantification for extracts with very low yields or samples with suboptimal purity ratios.

Protocol references

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