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# RNA extraction from cells, spheroids, or organoids encapsulated in alginate norbornene using Maxwell® RSC Plant RNA Kit

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Helen Kearney[1], Virginie Joris[1], Martyna Rydzyk[2], Lorenzo Moroni[1], Carlos Mota[1]

[1]MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, 6229 ER, Maastricht, the Netherlands;
[2]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

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 details the isolation of RNA from cells, spheroids, or organoids encapsulated in alginate norbornene hydrogels using the Maxwell® RSC Plant RNA Kit and automated Maxwell® RSC instrument. The cells, spheroids, or organoids are first released from the hydrogel matrix by alginate lyase digestion, followed by direct processing with kit reagents in the Maxwell® platform. The expected outcome is recovery of RNA with high yield and purity suitable for downstream applications such as qPCR and sequencing. Key optimizations include removal of the hydrogel prior to RNA extraction to reduce contamination from the matrix components, omission of the homogenization step to minimize contamination from residual matrix leftover, and adjustment of reagent volumes within the kit workflow to maximize RNA recovery in low-yield samples.

## Materials

Reagents

- Alginate lyase powder
- PBS (cell culture grade) or 0.1 M phosphate buffer (pH 6.3)

Maxwell® RSC Plant RNA Kit (Promega) – includes:

- Homogenization buffer
- Thiolglycerol
- Lysis buffer
- DNase solution
- Cartridges and plungers
- Elution tubes
- 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

### Equipment

- Tabletop centrifuge (capable of 300 g and 15,000 g at room temperature)
- Water bath (37 °C)
- Maxwell® RSC instrument (Promega)
- Spectrophotometer (e.g., NanoDrop) or fluorometer (e.g., Quantus/Qubit)
- –20 °C freezer
- –80 °C freezer
- Liquid nitrogen (for snap freezing, if samples not processed immediately)

## Safety warnings

- **Chemical hazards:** The kit contains **guanidine thiocyanate** and other chaotropic salts in lysis buffers. These are harmful if swallowed, inhaled, or in contact with skin.
- **PPE:** Always wear **lab coat, gloves, and safety goggles**. Avoid inhalation of powders and contact with skin or eyes.
- **Ventilation:** Perform sample handling and buffer preparation in a **well-ventilated area**.
- **Waste disposal:** All waste containing guanidine salts must be collected separately as **hazardous waste**. Do not mix with bleach-containing solutions (toxic gases may form).
- **Instrument use:** The Maxwell RSC instrument is fully enclosed and minimizes exposure risk during RNA isolation. Ensure cartridges are **loaded correctly** to avoid leaks or splashes.

## Alginate Norbornene Digestion

45m

1 Make 20 U/mL alginate lyase solution. 10m

1.1 Weigh 2 mg alginate lyase powder per 1 mL 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 cells/spheroids/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. For monolayer cultures a longer spin (300 g for 5 min) is recommended. A visible cell pellet should form at the bottom of the Eppendorf tube before proceeding to the next step.*

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 cells/spheroids/organoids twice with PBS to remove residual enzyme. Spin down at same speed setting used in step 6. 5m

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

## RNA isolation using Promega Maxwell® RSC Plant RNA kit and Maxwell® RSC instrument 1h 24m

10 Prepare Thiolglycerol/Homogenization buffer according to manufacturer's guidelines: 20 µL Thiolglycerol per 1 mL Homogenization buffer. Keep solution cold on ice. 5m

11 Retrieve cell/spheroid/organoid samples and place on ice. Immediately add 220 µL Thiolglycerol/Homogenization buffer. Allow samples to thaw in buffer. Gently invert samples twice. Do not pipette, shake, or homogenize samples! Keep samples cold on ice for 10 min. 10m

*Note: See the manufacturer's user guide for details on the recommended sample weight per RSC cartridge. The Maxwell® RSC Plant RNA kit uses the same reagents as the Maxwell® RSC simplyRNA Cells and simplyRNA Tissue kits. According to the simplyRNA Cells and simplyRNA Tissue kit manual, one RSC cartridge can process up to 5 million cells or 10 mg of tissue. Since our protocol uses human-derived material rather than plant material, we referred to the simplyRNA kit guidelines when determining input amounts.*

12 Add 220 µL lysis buffer to each sample. Gently invert twice. Do not pipette, shake, or homogenize! Incubate at room temperature for 10 min. 10m

13 While samples are incubating, prepare RSC Plant RNA kit cartridges:

*Note:* All reagents required for step 13 are provided in the Maxwell RSC Plant RNA kit.

13.1 Add 5 µL DNase solution in the dedicated well/compartment.

13.2 Prepare elution tube with 50 µL nuclease-free water

13.3 Place plunger in appropriate compartment

14 Transfer 400 µL lysed sample to a new Eppendorf tube. Spin at 15,000 g for 2 min at room temperature. 2m

15 Transfer the supernatant to the first well of the RSC cartridge, leaving behind any solid debris (if any) at the bottom of the Eppendorf tube. 2m

16 Run the Plant RNA kit protocol on the Maxwell® RSC instrument using the provided Maxwell® software. 45m

17 After the run finishes, remove the RNA elution tubes from the instrument and measure RNA yield using a Spectrometer or Fluorometer. 10m

*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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