RASTRUM

Protocol

RNA extraction from RASTRUM™ Advanced Cell Models using Promega ReliaPrep™ System

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Introduction

RNA extraction is a critical step for downstream applications such as RT-qPCR and RNA sequencing. While it can be performed after cells retrieval from the well plate using the Cell Retrieval protocol, the extraction can be simply performed by direct cell lysis into the cell well by adding BL+TG buffer (Promega ReliaPrep™ System). This method is particularly useful for sensitive cell types and disease models, where the cell integrity may be compromised and cannot sustain any additional steps of cell isolation. Herein, we provide an easy-to-follow protocol to extract high quality and purity RNA from a RASTRUM Large Plug Model, fulfilling the requirement for RT-qPCR or RNA sequencing downstream analysis. This protocol can extract RNA (100 ng/µl - 200 ng/µl) with 260/280 ratio >2.

Equipment and reagents required, but not provided

- RASTRUM 3D cell models (Large Plug)
- Promega ReliaPrep™ System (#Z6010)
- Dulbecco's phosphate-buffered saline (DPBS)
- Plate shaker
- Multichannel pipette
- Microcentrifuge (12,000 - 14,000g)
- Gloves
- Filtered tips

Note: Following Promega's manufacturing instruction for preparation and storage of BL+TG buffer is necessary for an efficient and a high quality of RNA extraction. The following steps should be performed in an RNAse free environment and appropriate experimenter PPE (clean lab gowns and gloves).

Protocol

1. Remove the media from the wells containing RASTRUM 3D cell model and wash each well with 100 µL of 1xDPBS for 5 min at room temperature.
2. Remove the 1xDPBS and add 100 µl of BL + TG Buffer to each well.
3. Lid the plate and place it on an orbital shaker at 200 rpm at room temperature (30 mins for 0.7 kPa and 1.1 kPa; 40 mins for 3 kPa) to promote cell lysis.
4. Collect the lysate from 6 wells to one microcentrifuge tube.

Caution: The lysis buffer will NOT degrade the matrix. Avoid vigorous pipetting and be mindful not to pierce through the matrix with the pipette tip, as this can cause the matrix to break into large pieces and potentially clog your column in subsequent steps.
Note: The porosity of the matrix and the shaking step allow the lysate to easily move out of the matrix and into the well. To maximise lysate removal and prevent piercing the matrix, tilt the plate at a 45-degree angle and gently pipette up and down several times before collecting the liquid at the bottom edge of the well.

5. Add 210 µl (35 µL x 6 wells) of isopropanol into each tube.

   Note: The volume of 35 µL was used based on table 1 in the ReliaPrep™ System protocol for a total of ~3 x 10^5 –5 x 10^5 cells in one tube from step 4. Adjust as per amount of predicted cells per tube.

6. Vortex each tube for 5 seconds.
7. Unpack one Minicolumn, two Collection Tubes and one Elution Tube for each sample from the Promega ReliaPrep™ System kit. Label each tube and Minicolumn on top and side. Place one Minicolumn into a Collection Tube for each sample.
8. Transfer lysate from Step 6 to a Minicolumn in a Collection Tube. and centrifuge at 12,000–14,000 × g for 30 seconds at 20-25°C.
9. Remove the ReliaPrep™ Minicolumn and discard the liquid in the Collection Tube. Replace the Minicolumn in the Collection Tube. Add 500 µl of RNA Wash Solution to the Minicolumn. Centrifuge at 12,000–14,000 × g for 30 seconds. Empty the Collection Tube.
10. Prepare DNase I incubation mix by combining the following amounts of reagent per sample (see table below). The volumes listed below make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare. Mix by gently pipetting up and down (do not vortex).

Table 1. Volume preparation of DNase I mix.

DNase I mix	1X
Yellow Core Buffer	24 µl
$MnCl_2$ (0.09M)	3 µl
DNase I	3 µl

11. Apply 30 µl of DNase I incubation mix to the Minicolumn membrane. Incubate for 15 min at 20–25°C.
12. Add 200 µl of Column Wash Solution (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds.
13. Add 500 µl of RNA Wash Solution (with ethanol added). Centrifuge at 12,000–14,000 × g for 30 seconds. Discard the wash solutions and the Collection Tube.
14. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add 300 µl of RNA Wash Solution and centrifuge at high speed for 2 min.
15. Transfer the ReliaPrep™ Minicolumn from the Collection Tube to an Elution Tube. Add 15 µL of Nuclease-Free Water to the Minicolumn membrane as recommended by Promega. Place the Minicolumn and Elution Tube into a centrifuge with the Elution Tube lid facing to the outside. Centrifuge at 12,000–14,000 × g for 1 min.

    Note: We used 15 µL of nuclease free water to align with the lysis buffer step regarding starting cell volume.

16. Discard the Minicolumn. Cap the Elution Tube containing the purified RNA and store at -80°C.
17. The RNA samples are ready for downstream analysis i.e RNA quantification, RT-qPCR, RNA sequencing, etc.

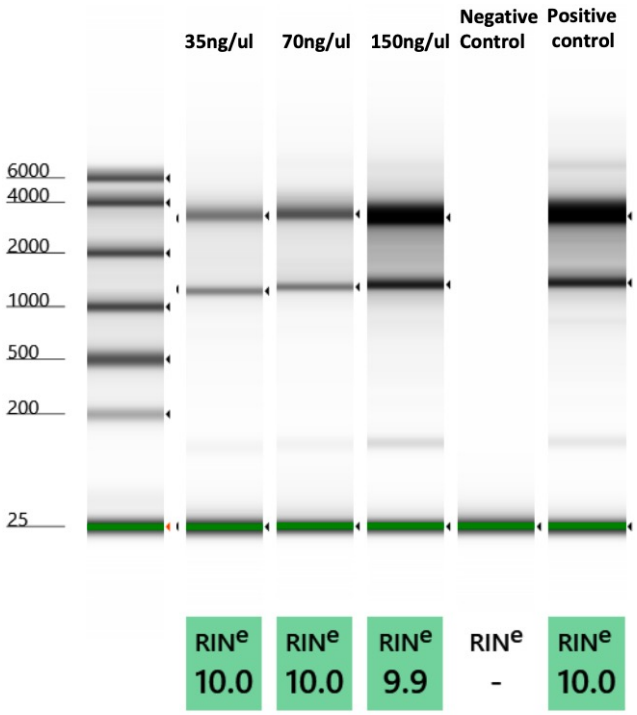

Figure 1: **Sample RNA integrity number.**
RNA extracted from RASTRUM advanced models meet the standard of RIN (>8) for downstream RNA sequencing 1. Our matrix production is also contamination free, no RNA is found in the matrix. Positive control = RNA extraction from 2D cultured cells, Negative control = RNA extraction from cell-free RASTRUM matrix. (The experiment was performed by Garvan Institute of Medical Research).

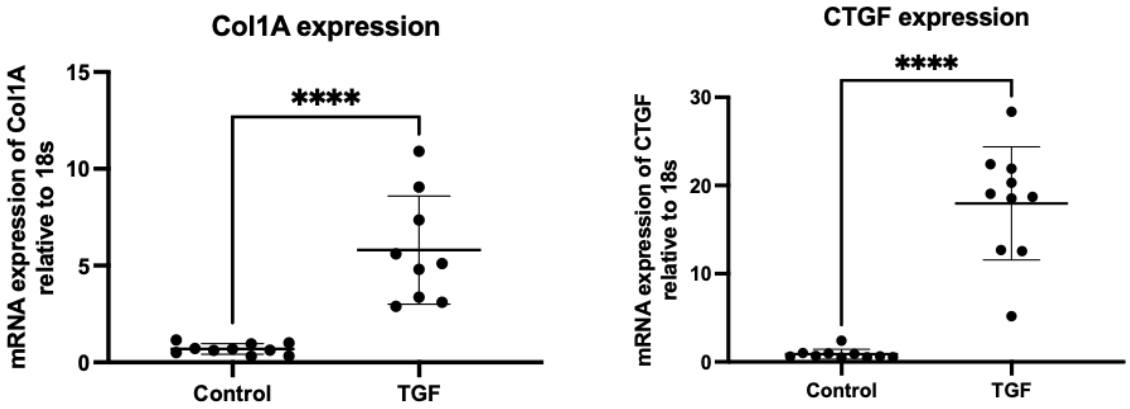

Figure 2: **Sample qPCR results.**
High quality RNA extracted from RASTRUM advanced liver fibrosis model (Co-culture of LX-2 and Ph5 cells) can reproduce accurate qPCR results. In this model, TGF-β1 treatment increased RNA expression of Collagen-1A and Connective tissue growth factor (CTGF), both are standard markers in liver fibrosis induction. ($p<0.0001$, TGF vs Control). n=2 biological replicates, with 9-10 technical replicates per experiment.

Reference

1. Westhaus A, Cabanes CM, Dilworth KL, Zhu E, Salas D, Navarro RG, Amaya AK, Scott S, Kwiatek M, McCorkindale AL, Hayman TE, Frahm S, Perocheau D, Tran BM, Vincan E, Wong SL, Waters SA, Riddiough GE, Perini MV, Wilson LOW, Baruteau J, Diecke S, Gonzalez-Aseguinolaza G, Santilli G, Thrasher AJ, Alexander IE, Lisowski L. *Assessment of pre-clinical liver models based on their ability to predict the liver-tropism of AAV vectors. Hum Gene Ther. 2023 Mar 16. doi: 10.1089/hum.2022.188. Epub ahead of print. PMID: 3692714*

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