



Total RNA isolation from >50,000 cells

Isolating Total RNA from large numbers of cells (>50,000) can create technical challenges for many extraction methodologies. Cell debris, large volume of liquids, large amounts of nucleic acid can all present unique problems depending on the technique being employed to perform the RNA extraction and purification.

Here we present a supplemental method utilizing SPRI technology to effectively and efficiently isolate total RNA from large cell numbers.

Research labs performing RNA based experiments with cultured cell lines or primary cells may use this protocol.

Please reference the current RNeasy Blood protocol for product information and a detailed description of use (Product Number: A35603, A35604 or A35605).

Purpose

The extraction of RNA from cultured cells can be used for different downstream applications like real-time PCR, RNA microarray, Northern Blot, RNA sequencing, etc.

Material needed

Description	Supplier	Part Number
Ethanol	American Bioanalytical	AB-00138
DNase I (RNase-free)	ThermoFisher Scientific Ambion	AM2222 or AM2224
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
Tube Magnet (1.5, 1.7, and 2ml)	A29182	Beckman Coulter

Protocol

1. Prepare
 - a. Remove cell culture media completely and wash the cells with 1xPBS one time.
 - b. Resuspend cells in **400 µL** of **1xPBS** per well.
2. Lysis
 - a. Add **300 µL** of Lysis Buffer and **20 µL** of Proteinase K.
 - b. **Mix** thoroughly by pipetting up and down 10 times.
 - c. **Incubate** samples at **37°C** for **15 minutes**.
3. Bind
 - a. Add **410 µL Bind 1/Isopropanol Solution** to the samples.
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. **Incubate** at **room temperature** for **5 minutes**.
 - d. Place the sample on a magnet for 10 minutes (or until the beads are settle).
 - e. Fully remove supernatant from the processing plate and discard.

4. Wash
 - a. Wash the beads by adding **800 μ L** of **Wash Buffer**.
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. Place the sample on a magnet for 10 minutes (or until the beads are settle).
 - d. Fully remove supernatant from the processing plate and discard.
5. Ethanol Wash
 - a. Add **800 μ L** of **80% ethanol** to the plate.
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. Place on magnet for 3 minutes (or until the beads are settled).
 - d. Fully remove supernatant from the plate and discard supernatant.
 - e. Remove plate from magnet.
6. DNase I treatment
 - a. Add **100 μ L** of **DNase solution**.
 - **80 μ L** of **Nuclease free water**
 - **10 μ L** of **DNase**
 - **10 μ L** of **DNase Buffer**
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. **Incubate** at **37 °C** for **20 minutes**.
7. Bind 2
 - a. Add **200 μ L** of **Bind 2 Buffer** to the plate.
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. **Incubate** at **room temperature** for **5 minutes**.
8. Ethanol Wash
 - a. Add **800 μ L** of **80% ethanol** to the plate.
 - b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
 - c. Place on magnet for 3 minutes (or until the beads are settled).
 - d. Fully remove supernatant from the plate and discard supernatant.
 - e. Remove plate from magnet.
9. Elute
 - a. Add **40 μ L** of **nuclease free water** to the plate.
 - b. **Incubate** at **60 °C** for **2 minute** while shaking at **300 rpm**.
 - c. Place on magnet for **5 minutes**.
 - d. Remove and **save** the supernatant without disrupting the beads.

Example Data

RNA was extracted from cell numbers ranging from 0.5 million to 3 million of colorectal carcinoma cell line HCT116. RNA yield was measured by Quant-iT™ RiboGreen® RNA Reagent (Thermo Fisher Scientific) (Figure 1). RNA yield increased in the cell number depended manner. About 60 µg of RNA was extracted from 3 million cells.

RNA integrity was also accessed using RNA ScreenTape assay (Agilent); all cell number groups showed high RNA integrity as represented by RIN scores (RIN: 9) (Figure 2).

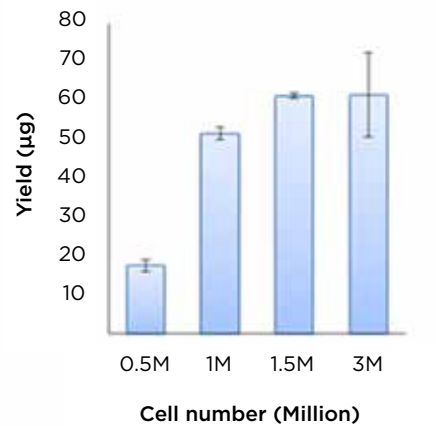


Figure 1. RNA yield from wide arrange of cell numbers. HCT116 was used in this experiment.

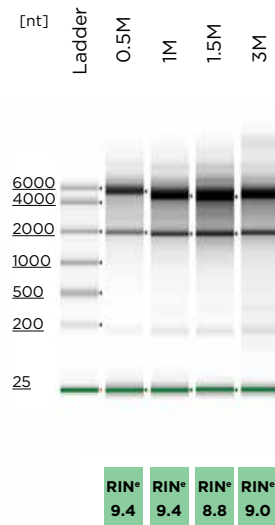


Figure 2. High RNA integrity was obtained by using RNAAdvance Blood supplementary protocol.

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