# [General protocol] RNA Extraction from Cultured Primate iPSCs

Author: Anne Hartleib
No tags associated

Created: 10.04.2023 12:50
Last modified: 14.04.2023 14:46

No custom dates added

General Protocol for RNA Extraction from Cultured Primate iPSCs
Version: 1.0 (10.05.2023)

**Media and Reagents:**
PBS-/-
70% ethanol
RNase AWAY Decontaminant
All other media and reagents are found in the RNeasy Mini Kit

**Materials and Equipment:**
Qiagen RNeasy Mini Kit

- Buffer RPE is supplied as a concentrate. Before using the kit for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution and tick the box on the cap to indicate that ethanol has been added.

1000 µL micropipette (Cell culture hood)
1000 µL micropipette tips (Cell culture hood)
Microcentrifuge
Vortex Mixer

**1. Introduction and Purpose**
This protocol describes the procedure for extracting total RNA from primate iPS cells cultured in multiwell plates using the Qiagen RNeasy Mini Kit.

**2. Before starting the protocol**
If purifying RNA from cell lines rich in RNAses, β-mercaptoethanol (β-ME) must be added to Buffer RLT before use (10 µl β-ME per 1 ml Buffer RLT). Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.

Cells grown in a monolayer in well plates can be lyzed directly on the vessel, cells grown in a flask must always be trypsinized first.

The following table summarizes the specifications to use the RNeasy Mini Spin Column:

| | |
|---|---|
| Maximum binding capacity | 100 µg RNA |
| Maximum loading volume | 700 µl |
| RNA size distribution | RNA >200 nucleotides |
| Minimum elution volume | 30 µl |
| Maximum amount of starting material for animal cells | 1 x $10^7$ |

**3. Protocol for RNA Extraction from Cultured Primate iPSCs**

1. Take Buffer RLT into the cell culture room.
2. Aspirate the medium from the wells and wash once with 1 mL PBS-/-.
3. Pipette an appropriate volume of Buffer RLT according to the table below with a 1000 µL micropipette. Do thorough up-and-downs to lyse all cells and remove the clumps.
4. Pipette all solution into a 1.5 mL Eppi, label it with cell line information, and bring it to the MolBio lab.
Before using the RNA Working bench, wipe all the surfaces and equipment with RNase AWAY Decontaminant.
5. Homogenize the cell pellet by vortexing the Eppi for 1 minute.
6. Add 1 volume (same volume of Buffer RLT that was used) of 70% ethanol to the homogenized lysate, and mix well by pipetting.
7. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.
**Note:** If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.
8. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.
**Note:** After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.
9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.
10. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane.
**Note:** After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
11. Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.

12. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNAse-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x g (≥10,000 rpm) to elute the RNA.
13. If the expected RNA yield is >30 µg, repeat step 12 using another 30–50 µl RNAse-free water, or using the eluate from step 12 (if high RNA concentration is required). Reuse the collection tube from step 12.
**Note:** If using the eluate from step 12, the RNA yield will be 15–30% less than that obtained using a second volume of RNAse-free water, but the final RNA concentration will be higher.
14. Measure the concentration with NanoDrop and Qubit.

| Culture Vessel | Surface Area ($cm^{2)}$ | Number of Cells | Volume of Buffer RLT for direct lysis (µl) |
|---|---|---|---|
| 6-well Plate | 9.5 | $1 \times 10^6$ | 600 |
| 12-well Plate | 4 | $5 \times 10^5$ | 350 |
| 24-well Plate | 2 | $2.5 \times 10^5$ | 350 |
| 48-well Plate | 1 | $1 \times 10^5$ | 350 |
| 96-well Plate | 0.32-0.6 | $4–5 \times 10^4$ | 350 |
| 35 mm Dish | 8 | $1 \times 10^6$ | 600 |
| 60 mm Dish | 21 | $2.5 \times 10^6$ | 600 |
| 100 mm Dish | 56 | $7 \times 10^6$ | 600 |
| 145 mm Dish | 145 | $2 \times 10^7$ | 600 |
| T25 Flask | 25 | $3 \times 10^6$ | Trypsinization needed |
| T75 Flask | 75 | $1 \times 10^7$ | Trypsinization needed |

**4. Storage of the Extracted RNA**
Purified RNA may be stored at −30 to −15°C freezer or −90 to −65°C freezer in RNAse-free water. Under these conditions, no degradation of RNA is detectable after 1 year.