Title

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mRNA collection and isolation from cells

Protocol

Materials:

- Box with ice
- Phosphate Buffered Saline (PBS) (Catalog Nr: D8537, Sigma Aldrich, Co., stored at 2-8 °C)
- Tissue culture dish with cells
- 1,5 ml tubes
- TRI reagent (Cat. No. : T9424, Sigma Aldrich)
- Chloroform
- Isopropanol
- Ethanol 75%

Procedure:

Day 1: Preparation of mRNA samples out of cells monolayer
The first part, the preparation of RNA samples out of cells, should be performed under sterile workbench.

1. Aspirate medium and wash 2x with pre-warmed PBS (for 10 cm tissue culture dish use approx. 5 mL). After last wash, add 1 mL of the PBS which should cover the whole surface and scrape off cells using a cell scraper. Transfer the cell suspension into a 1.5 mL tube.
2. Count the cells
3. Centrifuge the cells for 5 min, 700 x g, 4°C in a microcentrifuge.
4. Carefully remove and discard supernatant and re-suspend the cell pellet in 1 mL (for 3 x 10^6 cells in 600 µL) TRI reagent by repeated pipetting.

If necessary, the samples are stable in the TRI reagent and can be stored at -80°C for up to one month.

Day 2: mRNA isolation, determination of mRNA concentration

For the following mRNA isolation, you can work outside of the sterile workbench. Due to the use of chloroform, further steps will be performed under a safety hood.

1. Place your samples on ice and equilibrate them gently to room temperature before proceeding.
2. Add 0.2 mL chloroform per mL of before added TRI Reagent. Close the lids and vortex for 15 sec. Incubate samples for 2 - 15 min at room temperature.
3. Centrifuge at 12'000 x g for 10 min at 4°C to accomplish phase separation. Meanwhile, prepare labelled 1.5 mL tubes.
4. Transfer the upper colorless aqueous phase to a fresh 1.5 mL tube. Note: Take care to not transfer little amounts of the white interphase or the red organic phase in order to avoid contamination.
5. Add 0.5 mL isopropanol per mL of before added TRI Reagent. Incubate samples for 5 - 10 min at room temperature.
6. Centrifuge at 12'000 x g for 10 min at 4°C. The precipitated RNA will form a pellet.

Note: The pellet can be very small. If the RNA pellet floats or a milky RNA layer forms on top of the liquid, repeat the centrifugation one more time.

7. Remove supernatant with a pipette or by spilling it into a waste container.
8. Wash pellet by adding at least 1 mL 75% EtOH per mL of before added TRI Reagent. Vortex briefly. Centrifuge at 7'500 x g for 5 min at 4°C. Repeat the washing for another two times.
9. Remove EtOH with a pipette and let the pellet dry by air or applying vacuum for 10 min using the Eppendorf Concentrator plus in D-AL mode with the tubes being open.
10. Add an appropriate volume of sterile water by pipetting up and down in order to dissolve the RNA pellet. If the pellet is visible, take (20) 30 – 60 µL. If it is not visible, take less. Note: use pre-warmed water (55-60°C) to ease solubilization.
11. Determine the concentration of your DNA samples on a Nanoquant-Plate with Tecan infinite M200 Pro measuring device. Therefore, measure sterile water as blank first. Use an amount of 2 µL per sample.
12. Prepare labelled PCR tubes. Add the calculated volume of your isolated RNA sample, which is necessary to obtain a final concentration of 100 ng/µL RNA in a total volume of 10 µL. Dilute with sterile water. The samples can be stored at -80°C overnight.