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RNA Isolation from Cultured Mammalian Cells Using TRIzol Reagent V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is intended to isolate total RNA from cultured mammalian cells using TRIzol reagent, facilitating downstream applications such as RT-PCR, Northern blotting, and gene expression analysis.

Materials

Materials -
TRIzol Reagent 5 mL
Chloroform 1.5 mL
Isopropanol 1.2 mL
Ethanol (75%) 1.5 mL
RNAse-free Water 1 mL
Microcentrifuge tubes 5 tubes
Pipette tips 5 tips
Cell scraper 1
Ice As needed

Reagents -
TRIzol Reagent
Chloroform
Isopropanol
Ethanol (75%)

Troubleshooting

Safety warnings

Always wear gloves, lab coat, and safety goggles when handling TRIzol, chloroform, and isopropanol. Work in a fume hood when using volatile organic solvents.

Cell Lysis and RNA Extraction

1 Remove the culture medium from the cells using a vacuum or pipette. Add 1 mL of cold TRIzol reagent to the cell culture dish. Scrape the cells into the TRIzol using a sterile cell scraper. Transfer the cell suspension to a 1.5 mL microcentrifuge tube on ice. Vortex the tube for 10 seconds to ensure complete lysis.

2 Add 0.2 mL of chloroform to the tube. Vortex for 15 seconds and let it sit at room temperature for 2-3 minutes. Centrifuge the tube at 12,000 g for 15 minutes at 4 °C.

RNA Precipitation

3 Recover the upper aqueous phase (approximately 0.5 mL) into a new microcentrifuge tube. Add 1.25 mL of isopropanol to the aqueous phase. Invert the tube several times to mix. Incubate the tube at -20 °C for at least 1 hour to precipitate RNA.

4 Centrifuge the tube at 12,000 g for 30 minutes at 4 °C to pellet the RNA. Discard the supernatant and wash the RNA pellet with 1 mL of 75% ethanol. Centrifuge again at 7,500 g for 5 minutes at 4 °C.

Protocol references

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