Systems Bioengineering Laboratory

RNA Isolation with TRIzol

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Materials and equipment

- TRIzol (Life Technologies; Cat# 15596018)
- Cell culture dish containing cells
- 1X PBS (Boston BioProducts; Cat# BM-220), chilled on ice
- Chloroform (Sigma-Aldrich; Cat# C2432)
- 2-Propanol/Isopropyl Alcohol (Sigma Aldrich; Cat# I9516)
- Molecular Biology Grade Ethanol (Sigma- Aldrich; Cat# E7023) 70%, chilled on ice
- Nuclease Free Water (New England Biolabs Catalog # B1500S), chilled on ice
- Refrigerated microcentrifuge
- 2 mL microcentrifuge tubes
- DNA LoBind 1.5 mL Eppendorf tubes (Fisher Scientific; Cat# 022431021)
- Ice bucket with ice

Method

Cool PBS on ice. Place Culture dish containing cells on ice.
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Discard medium. Quickly wash 2X with cold PBS.
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Add 1 mL TRIzol into 60 mm dish. Incubate in hood for ≥ 5 minutes.
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Lyse the cells directly in the culture dish by pipetting up and down several times
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Transfer cells to 1.5 mL tubes
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Centrifuge at 12,000g for 10 minutes at 4°C.
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Transfer cleared supernatant to a new tube. Discard pellet.
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Add 200 μL Chloroform to the tube (200 μL per 1 mL TRIzol).
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Shake the tube vigorously by hand for 30 seconds.
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Centrifuge at 12,000g for 10 minutes at 4°C.
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Transfer the supernatant to a new tube. Avoid disturbing the interphase.
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Add 500 μL 100% Isopropanol to each tube containing supernatant

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Centrifuge at 12,000g for 10 minutes at 4°C

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Remove supernatant, leaving only the RNA pellet.

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Wash pellet with 1 mL of 75% Ethanol (molecular biology grade). Pipette for 30 seconds

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Centrifuge at 7,500g for 10 minutes at 4°C.

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Discard the wash. Repeat washing steps twice.

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Discard wash. Air dry pellet for 10-15 minutes at room temperature

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Re-suspend RNA pellet in 20-30 μL RNAse-free water

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Quantify RNA on Qubit or Nanodrop

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Store at -80°C (long-term) or -20°C (short-term)