RNA Extraction from Cell Culture

References

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Protocol

1. Rinse cells 3X with sterile MilliQ water.
2. Scrape cells in Trizol (800 µL for 60 mm high density culture) using a sterile scraper.
3. Transfer homogenized sample into 1.5 ml sterile Eppendorf tube and incubate for 5 mins at room temperature to permit the dissociation of nucleoprotein complexes.
4. Add 160 µL of chloroform to separate RNA from DNA and proteins (phase separation).
5. Shake tubes vigorously by hand for 15 seconds (DO NOT VORTEX) and incubate for 3 minutes at room temperature.
6. Centrifuge the samples at 12,000 x g for 15 minutes at 4°C.
7. Transfer upper aqueous supernatant to a new sterile Eppendorf tube.
8. Add 400 µL of Isopropanol to precipitate RNA. Mix gently and incubate for 10 minutes at room temperature.
9. Centrifuge at 12,000 x g for 15 mins at 4°C.
10. Discard the supernatant (the pellet may be invisible: mark expected pellet location on tubes).
11. Wash the pellet in 800 µL of 75% RNase-free Ethanol.
12. Centrifuge at 7,500 x g for 5 minutes at 4°C.
13. Remove ethanol completely and re-suspend pellet in 10 µL of DEPC-H_20.
14. Record RNA concentrations and store at -80°C.