Extracting Total RNA

Introduction

Isolation of high-quality intact RNA at consistent yields is a critical step in many molecular biology-based procedures. Several extraction methods are available depending on the biological sample used and the amount of RNA needed for subsequent experiments. The protocol presented here has demonstrated efficacy and consistency in extracting total intact RNA in high quality and purity from iCell® Cardiomyocytes. The expected yield is ~1 - 1.5 µg of RNA per well of a 24-well cell culture plate (120,000 plated cells/well) when iCell Cardiomyocytes are cultured according to the User's Guide.

Required Equipment and Consumables

The following equipment and consumables are required in addition to the materials specified in the iCell Cardiomyocytes User's Guide.

Item	Vendor	Catalog Number
Equipment		
Heating Block, capable of maintaining 55˚C	Multiple Vendors	
Tabletop Centrifuge with Rotor for 50 ml or 2 ml Tubes	Multiple Vendors	
Consumables		
iCell Cardiomyocytes Kit	Cellular Dynamics International (CDI)	CMC-100-110-001 CMC-100-110-005 CMC-100-010-001 CMC-100-010-005
1.5 ml RNase-free Tubes	Multiple Vendors	
14.3 M β-mercaptoethanol	Multiple Vendors	
24-well Cell Culture Plates	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14190
Fibronectin	Roche Applied Science	11051407001
Molecular Biology Grade Ethanol	Multiple Vendors	
QIAGEN Proteinase K	Qiagen	19131
QIAshredder	Qiagen	79654 79656
RNeasy Micro Kit	Qiagen	74004
Sterile RNase-free Pipettor Tips	Multiple Vendors	
Sterile Water	Multiple Vendors	

Methods

Notes

Culturing iCell Cardiomyocytes

1. Dilute 1 mg/ml fibronectin solution in sterile D-PBS to a final concentration of 5 µg/ml immediately before use.

   Note: *Reconstitute fibronectin in sterile water at 1 mg/ml according to the manufacturer's instructions. Aliquot and store at -20˚C.*

2. Add 0.6 ml/well of the 5 µg/ml fibronectin solution to a 24-well cell culture plate.
3. Incubate in a cell culture incubator at 37°C overnight.
4. Thaw iCell Cardiomyocytes according to the iCell Cardiomyocytes User's Guide.
5. Dilute the iCell Cardiomyocytes cell suspension in iCell Cardiomyocytes Plating Medium to 200,000 plated cells/ml. See the iCell Cardiomyocytes User's Guide for instructions to calculate the *Target Plating Density* based on *Plating Efficiency*
6. Aspirate the fibronectin solution. Immediately add 0.6 ml/well of the cell suspension (120,000 plated cells/well).
7. Culture iCell Cardiomyocytes in a cell culture incubator at 37°C, 7% CO_2.
8. Maintain the cardiomyocytes according to the User's Guide until ready to perform total RNA extraction.

Extracting Total RNA from iCell Cardiomyocytes

The following procedure details extracting total RNA from iCell Cardiomyocytes cultured in 24-well cell culture plates. For other cell culture vessel formats, see the RNeasy Micro Kit's instructions.

1. Prepare the solutions required for total RNA extraction according to the RNeasy Micro Kit's instructions. When instructed, add β-mercaptoethanol instead of DTT to Buffer RLT.
2. Wash iCell Cardiomyocytes once with 0.5 ml/well of D-PBS.
3. Disrupt the cardiomyocytes by adding 150 µl of Buffer RLT directly onto the cell monolayer. Pipette to mix.
4. Homogenize the cardiomyocytes lysate by loading it onto a QIAshredder spin column placed in a 2 ml collection tube, and centrifuging at full speed for 2 minutes.
5. Transfer the cardiomyocytes lysate to a new 1.5 ml RNase-free tube. Dilute the cardiomyocytes lysate in 295 µl of RNase-free water. Add 5 µl of 20 mg/ml Proteinase K.
6. Incubate on a heated block at 55˚C for 10 minutes. Centrifuge the cardiomyocytes lysate at 10,000 x g at room temperature for 3 minutes.
7. Transfer the supernatant to a new 1.5 ml RNase-free tube without disturbing the pellet of cell debris. Add 0.5 volumes of 96 - 100% ethanol and mix well by pipetting.

Notes

8. Transfer the sample to an RNeasy MinElute spin column placed in a 2 ml collection tube and centrifuge at 10,000 x g for 15 seconds. Aspirate and discard the flow-through.
9. Add 350 µl of Buffer RW1 to the RNeasy MinElute spin column and centrifuge at 10,000 x g for 15 seconds. Aspirate and discard the flow-through.
10. Add 10 µl of DNase I stock solution to 70 µl of Buffer RDD and mix gently.
11. Add the 80 µl of DNase I mix directly to the RNeasy MinElute spin column membrane and place on the benchtop at room temperature for 15 minutes.
12. Add 350 µl of Buffer RW1 to the RNeasy MinElute spin column and centrifuge at 10,000 x g for 15 seconds. Aspirate and discard the flow-through.
13. Place the RNeasy MinElute spin column in a new 2 ml collection tube. Add 500 µl of Buffer RPE to the RNeasy MinElute spin column and centrifuge at 10,000 x g for 15 seconds. Aspirate and discard the flow-through.
14. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column and centrifuge at 10,000 x g for 2 minutes. Discard the collection tube with the flow-through.
15. Place the RNeasy MinElute spin column in a new 2 ml collection tube and centrifuge at full speed for 5 minutes. Discard the collection tube with the flow-through.
16. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube. Add 14 µl of RNase-free water and centrifuge at full speed for 1 minute. Discard the RNeasy MinElute spin column.
17. Store the eluted total RNA at -20°C to -70°C.

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