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ArrayPure™ Nano-scale RNA Purification Kit

Cat. No. MPS04050

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1. Introduction

The ArrayPure™ Nano-scale RNA Purification Kit provides all of the reagents needed to purify RNA from ten to a few hundred eukaryotic cells, such as obtained from Laser Capture Microdissection procedures. The reagents are all aqueous to avoid the use of toxic organic solvents.[1] This nano-scale protocol has been developed for 1-10,000 eukaryotic cells.[2]

2. Product Specifications

Storage: Store the Proteinase K and RNase-Free DNase I at –20°C in a freezer without a defrost cycle. The rest of the kit may be stored at room temperature for ease of use.

Storage Buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 10 mM $CaCl_2$. Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100, and 1 mM dithiothreitol (DTT).

Quality Control: The ArrayPure Nano-scale RNA Purification Kit is function-tested by purifying RNA from HeLa cells. RNA quality and yield are assayed by fluorimetry and used as a template for RT-PCR.

Note: *The ArrayPure Nano-scale RNA Purification procedure is incompatible with tissue samples prepared with RNAlater® or RNAlater-ICE.*

3. Kit Contents

Desc.	Concentration	Quantity
The ArrayPure Nano-scale RNA Purification Kit is available in a 50-purification size. The kit contains:		
Nano-scale Lysis Solution		1.5 ml
2X Nano-scale Lysis Solution		1.0 ml
MPC Protein Precipitation Reagent		1.9 ml
Proteinase K	@ 5 µg/µl	50 µl
RNase-Free DNase I	@ 1 U/µl	25 µl
RiboGuard™ RNase Inhibitor	@ 40 U/µl	100 µl
1X DNase Buffer (33 mM Tris-HCl [pH 7.8], 66 mM potassium acetate, 10 mM magnesium acetate and 0.5 mM DTT)		1.0 ml
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		500 µl

4. Related Products

The following products are also available:

– MasterPure™ RNA Purification Kit

5. Nano-Scale RNA Purification Protocol

A. RNA Purification

1. Dilute 1 µl of 5 µg/µl Proteinase K into 30 µl of Nano-scale Lysis Solution for each sample. A premix may be prepared for multiple samples. Scale-up in 30-µl increments is possible. Skip to Step 4 if the cells are adhered to an LCM cap.
2. Pellet 1-10,000 cells by centrifugation for 5 minutes at 3,500 x g and discard the supernatant. Wash the cells by resuspending the cells in 200 µl of phosphate buffered saline and centrifuge again. Discard supernatant.
3. Repeat step 2.
4. Add 30 µl of Nano-scale Lysis Solution containing the Proteinase K to the tube containing the captured cells or cell pellet; mix thoroughly by vortexing.
5. Incubate at 65-70°C for 15 minutes.
6. Place the samples on ice for 3-5 minutes. Then add 18 µl of MPC Protein Precipitation Reagent to 30 µl of lysed sample (solution may become cloudy). Vortex vigorously for 10 seconds.
7. Pellet the debris by centrifugation for 7 minutes at 4°C at ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
9. Add 50 µl of isopropanol to the recovered supernatant. Vortex briefly.
10. Pellet the RNA by centrifugation at 4°C for 5 minutes at ≥10,000 x g in a microcentrifuge. The pellet will be invisible. Orient the tube in the microcentrifuge so that you will know where the pellet is.

B. Removal of Contaminating DNA from RNA Preparations

1. Remove all of the residual isopropanol with a pipet. Allow the pellet to air dry for 5 minutes.
2. Prepare 20 µl of DNase I solution for each sample. Add 1 µl of RNase-Free DNase I to 40 µl of 1X DNase Buffer.

   Note: *The 40 µl is enough for 2 samples.*
3. Completely resuspend the nucleic acid pellet in 20 µl of DNase I solution.
4. Incubate at 37°C for 10 minutes.

   Note: *Incubation up to 30 minutes may be necessary to remove all contaminating DNA.*
5. Add 20 µl of 2X Nano-scale Lysis Solution; vortex mix for 5 seconds.
6. Add 20 µl of MPC Protein Precipitation Reagent (solution may become cloudy). Vortex mix 10 seconds; place on ice 3-5 minutes.
7. Pellet the debris by centrifugation at 4°C for 5 minutes at ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 50 µl of isopropanol to the supernatant. Vortex briefly.

10. Pellet the purified RNA by centrifugation at 4°C for 5 minutes in a microcentrifuge at ≥10,000 x g.
11. Carefully aspirate the isopropanol without dislodging the RNA pellet, which is invisible.
12. Rinse once with 70% ethanol, being careful to not dislodge the pellet. Centrifuge for 3 minutes at ≥10,000 x g. Remove all of the residual ethanol with a pipet. Allow the pellet to air dry for 5 minutes.
13. Resuspend the RNA in 5-10 µl of TE Buffer.
14. Add 1 µl of RiboGuard™ RNase Inhibitor.

6. References

1. Miller, S.A. *et al.*, (1988) *Nucl. Acids Res.* **16**, 1215.
2. Jarvis, B. W. (2004) *Epicentre Forum* **11**, (6), 5.

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