Agencourt® RNAClean™ XP

SUPPLEMENTAL PROTOCOL FOR Micro RNA CLEAN UP USING AGENCOURT® RNAClean® XP

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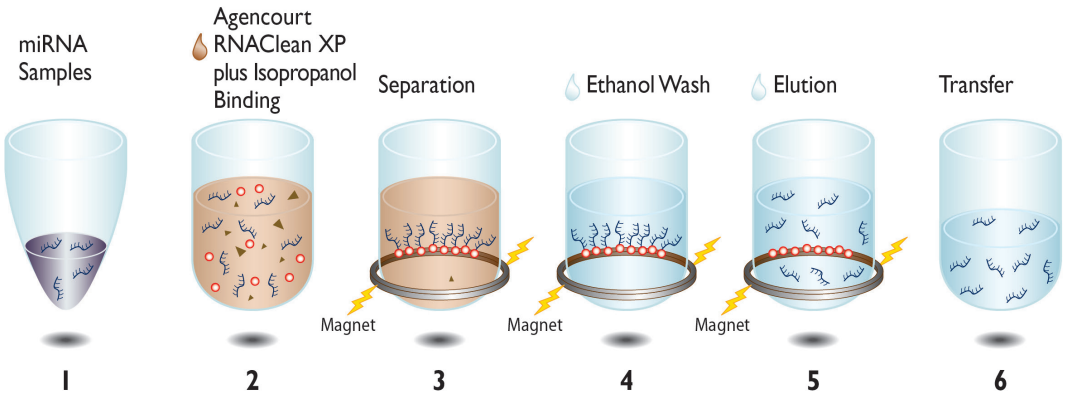
PROCESS OVERVIEW



Introduction

The Agencourt RNAClean XP system utilizes Beckman Coulter's solid-phase paramagnetic bead technology for high-throughput purification of RNA or cDNA for in vitro applications such as transcription, antisense RNA (aRNA) amplification as well as RNA and cDNA probe synthesis. This supplemental protocol enables recovery of micro RNA (miRNA), small RNA and total RNA from enzymatic reactions, concentrating miRNA and total RNA from a diluted sample.

Materials Supplied by the User

Consumables and Hardware:

Magnetic Separator: Agencourt SPRIPlate 96R-Ring Super Magnet Plate: Beckman Coulter Life Sciences, A32782) or Agencourt SPRIStand-Magnetic 6-tube Stand (for 1.7 mL tubes) (Beckman Coulter Life Sciences, A29182)

Prep Plate: ABgene 1.2 mL 96-Well Storage Plate, Square Well, UBottom (ABGene # 1127).

Reagents:

- 100% Isopropanol: American Bioanalytical, AB07015
- 100% ethanol: American Bioanalytical, AB00138
- 85% ethanol made with nuclease free water (Note: 85% ethanol is hygroscopic. *Fresh 85% ethanol should be prepared for optimal results).*
- Nuclease-Free Water (not DEPC-Treated) (Ambion, AM9932)

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Procedure

Note: Shake the Agencourt RNAClean XP bottle to re-suspend any magnetic particles that may have settled.

1. Add the appropriate volume of RNAClean XP and 100% Isopropanol (American Bioanalytical, part # AB07015) into the well containing 50-200 µL of miRNA sample according to the instructions in the table below.

SAMPLE VOLUME (µL)	RNAClean XP (µL)	100% ISOPROPANOL (µL)
50	90	270
100	180	270
150	270	270
200	360	270

2. Mix the total reaction volume by pipetting up and down 10 times and incubate at room temperature for 5 minutes. Place the reaction vessel on an Agencourt SPRIPlate 96R-Ring Super Magnet Plate or Agencourt SPRIStand-Magnetic 6-tube and allow the RNAClean XP beads to settle on the magnet for 15 minutes or until the supernatant turns completely clear.
3. Remove and discard the entire clear supernatant.
   NOTE: *Care should be taken not to aspirate a significant amount of beads during this step as the desired miRNA and total RNA is associated with the beads. Significant bead loss will result in reduced yield.*
4. With the reaction vessel still on the magnet, add 600 µL of 85% nondenatured ethanol (freshly made from 100% ethanol) and incubate at room temperature for 30 seconds.
5. Remove and discard the ethanol supernatant.
   NOTE: *Care should be taken not to aspirate a significant amount of beads during this step as the desired miRNA and total RNA is associated with the beads. Significant bead loss will result in reduced yield.*
6. Repeat steps 4-5 with the ethanol wash for a total of 2 ethanol washes. Air dry the beads for 5 minutes.
7. To elute the sample, remove the reaction vessel from the magnet and add 20-50 µL of molecular biology grade RNase/DNase free water or 10 mM Tris-HCl, pH8.5.
   NOTE: *Elution volume should be large enough so that the liquid level is high enough on the magnet for the beads to settle.*
8. Mix the total elution volume by pipetting up and down 10 times to resuspend the beads and incubate at room temperature for 2 minutes.
9. Place the reaction vessel on an appropriate magnetic stand or plate and allow the RNAClean XP beads to settle to the magnet for 2 minutes.
10. Transfer the eluted samples to an appropriate storage vessel and keep at -80˚C for long term storage.

For more information, visit www.Beckman.com or contact 1-800-369-0333