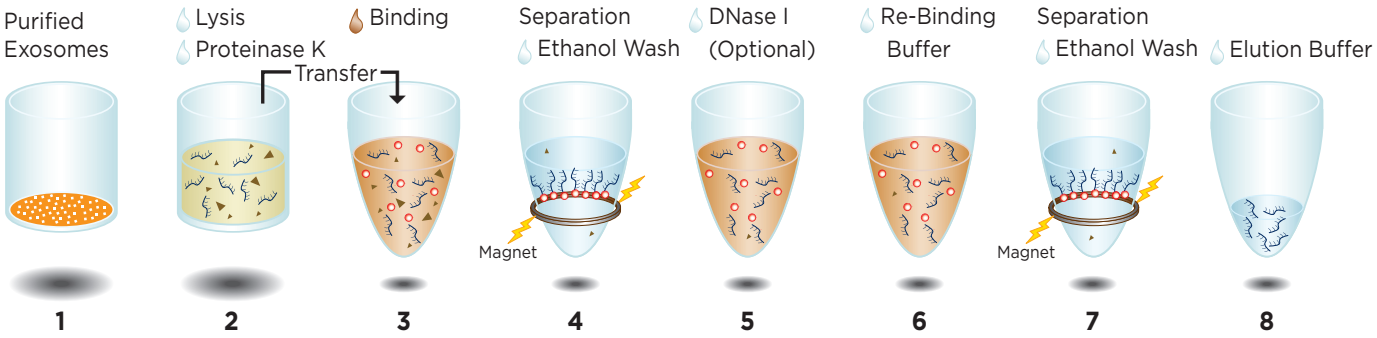
Agencourt® RNAdvance® CELL v2 Kit Supplemental Protocol for miRNA and total RNA Isolation from Exosomes

Process Overview



Introduction

The Agencourt RNAdvance Cell v2 purification kit utilizes Beckman Coulter's patented Agencourt SPRI® paramagnetic bead-based technology to isolate total RNA from cultured eukaryotic cell lines. The protocol is modified to isolate micro RNA (miRNA) and total RNA from 10-50 µL of exosomes isolated from cultured medium or body fluids in 96 well or 1.7 mL tube formats.

Notice for miRNA and total RNA Isolation

- If it is planned to use the RNAdvance Cell v2 kit for miRNA plus total RNA isolation, **100% Isopropanol must not be added directly to the Wash Buffer bottle**. See Reagent Preparation, step 4 for wash buffer preparation. A 1:2 ratio of Wash buffer: 100% Isopropanol is used for miRNA plus total RNA isolation.
- Binding buffer conditions: 80µL of Bind buffer + 250µL of Isopropanol. Use 330µL in the Binding step.
- Use 85% ethanol for all ethanol washing steps.

Materials Supplied by the User

Consumables and Hardware:

- Agencourt SPRIPlate 96R – Ring Super Magnet Plate (Beckman Coulter Life Sciences, A32782) or Agencourt SPRIStand – Magentic 6-tube Stand (for 1.7 mL tubes) (Beckman Coulter Life Sciences, A29182)
- ABgene 1.2mL 96-Well Storage Plate, Square Well, U-Bottomed (ABGene #1127) for plate format
- 1.7 mL microcentrifuge tubes (Fisher Scientific, NC9448938) for tube format

Reagents:

- 100% Isopropanol; American Bioanalytical AB07015 or equivalent
- 85% ethanol; freshly prepared/diluted from 100% ethanol (American Bioanalytical, AB00138 or equivalent)
- DNase I (RNase-free); Ambion AM2222 or AM2224
- Reagent grade water, nuclease-free; Ambion AM9932

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General Remarks on Handling RNA

RNases are ubiquitous and general precautions should be followed in order to avoid their introduction to the sample during the Agencourt RNAdvance Blood procedure. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions and glassware. When working with RNA, the following procedures should be followed to limit RNase contamination:

- Always work with gloved hands and change gloves frequently.
- Use RNase free, filtered pipette tips for pipetting whenever possible.
- Use dedicated RNase free equipment, e.g. pipettes, pipette tips, gels boxes, etc.
- Avoid using reagents, consumables and equipment that are in common use for other general lab processes.
- When available, work in a separate room, fume hood or lab space.
- Use plastic, disposable consumables that are certified RNase free.
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free.
- Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contaminating the stock solution.
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 85% ethanol before starting work.

Reagent Preparation

1. For each new kit, assemble Proteinase K once. Mark each tube or bottle with the date of assembly. Mix components by inverting the tube/bottle several times. To avoid foaming, do not vortex. The solution will appear cloudy immediately after mixing – let the solution sit for 5 minutes to clear prior to using. **Store the Proteinase K solution at -20° C when not in use.**

	Proteinase K Solution (50 mg/mL) Volume of PK Buffer to add to lyophilized Proteinase K
96 Prep kit #001354 / A47942	400 µL
960 Prep kit #001355 / A47943	4 mL
Storage Condition	-20˚C

2. Wash Buffer Preparation:

For miRNA and total RNA isolation: Add 100% Isopropanol to the Wash Buffer in a proportion of 1:2 (Wash Buffer: isopropanol). To make 10 mL of wash buffer solution, add 6.67 mL of 100% Isopropanol with 3.33 mL of Wash Buffer in a 15 mL conical tube and vortex thoroughly for 10 seconds.

For total RNA isolation only: Add 100% Isopropanol to the Wash Buffer in a proportion of 1.5: 1 (Wash Buffer: isopropanol). To make 10 mL of wash buffer solution, add 4 mL of 100% Isopropanol with 6 mL of Wash Buffer in a 15 mL conical tube and vortex thoroughly for 10 s.

3. Prepare fresh 85% ethanol with nuclease free water. (Note: 85% ethanol is hygroscopic. Fresh 85% ethanol should be prepared for optimal results).

4. Prepare Bind Buffer Solution – Prepare this solution fresh and discard any unused solution.

330 µL of Bind/Isopropanol solution are required per sample. Vortex the tube containing the Bind Buffer magnetic beads for at least 30 seconds to fully resuspend the beads. Combine 80µL Bind Buffer with 250 µL 100% isopropanol and mix thoroughly.

5. Prepare DNase Solution – Prepare this solution fresh-discard any unused solution.

25 µL of 1X DNase solution are required per sample. Combine 20 µL nuclease-free water with 2.5 µL 10X DNase buffer, and 2.5 µL of DNase I and mix thoroughly by pipetting or inverting the tube.

6. Prepare Lysis/PK Solution – USE WITHIN 30 MINUTES.

63 µL of Lysis/PK of solution are required per sample. Combine 3 µL PK (50 mg/mL) with 60 µL of Lysis Buffer, for a total of 63 µL Lysis/PK Solution. Mix gently to avoid creating bubbles.

Procedure

1. Add 63 µL of Lysis/PK Solution (see Reagent Preparation, step 6) **to each sample either in a tube or 96-well plate.** Gently pipette tip mix 10 times at the bottom of the well to resuspend the exosomes suspension.

2. Incubate the samples for 30 minutes at room temperature to complete the lysis and digestion.

3. Add 330 µL of Bind Buffer Solution to each sample and pipette tip mix 10 times or until homogeneous.

4. Incubate the samples for 5 minutes at room temperature to bind nucleic acids.

5. Place the sample plate on an Agencourt SPRIPlate 96R – Ring Super Magnet Plate or Magnetic Stand for 5 minutes or wait for the solution to turn completely clear.

Carefully aspirate and discard the supernatant while the plate is situated on the magnet.

When aspirating, place the pipette at the center of the well to avoid disturbing the magnetic beads.

6. Take the plate off the magnet. Add 300 µL of Wash Buffer. (See Reagent Preparation, step 4.) **Pipette tip mix 10 times, or until the magnetic particles are fully resuspended.**

It is normal for a few bead clumps to remain after resuspension.

7. Place the plate back on the magnet for 5 minutes, or wait for the solution to turn completely clear. Fully remove and discard the supernatant while the plate is situated on the magnet.

When aspirating, place the pipette at the center of the well to avoid disturbing the magnetic beads.

8. Take the plate off the magnet. Add 300 µL of 85% ethanol. Gently pipette tip mix 5 times, or until beads are fully resuspended.

9. Place the plate back on the magnet for 5 minutes, or wait for the solution to turn completely clear. Thoroughly remove and discard as much of the ethanol wash as possible.

Excess ethanol can reduce the activity of DNase during the next steps.

OPTIONAL DNAse treatment: Skip steps 11-15 if DNase treatment is not required.

11. Take the plate off the magnet. Add 25 µL of DNase Solution (see Reagent Preparation, step 5) **and pipette tip mix 10 times, or until the beads are fully resuspended.**

The addition of aqueous DNase releases DNA and RNA from the beads. DNA will be digested and the RNA will need to be re-bound to the beads later in the protocol.

12. Incubate the sample plate at room temperature for 15 minutes to complete the DNase digestion.

13. DO NOT REMOVE THE DNase SOLUTION. Add 165 µL of Wash Buffer to each sample and pipette tip mix 10 times, or until homogeneous.

During this step, Wash Buffer re-binds RNA to the beads. Additionally, the Wash Buffer helps to dissolve and rinse away proteins and other contaminants.

14. Incubate the plate at room temperature for 5 minutes to bind.

15. Place the plate on the magnet for 5 minutes or wait for the solution to turn completely clear. Remove and discard the supernatant.

16. Take the plate off the magnet. Wash the beads by adding 300 µL of 85% ethanol.

Pipette tip mix 5 times, or until beads are fully resuspended.

Ethanol washes remove salt, Wash Buffer and any residual contaminants.

17. Place the sample plate on the magnet for 5 minutes or wait for the solution to turn completely clear. Remove ethanol and discard.

18. Repeat steps 16-17 one more time for a total of 2 ethanol washes.

19. Remove as much of the final ethanol wash as possible. Allow the beads to dry for 3-5 minutes at room temperature while the sample plate is on the magnet.

Any droplets or puddles of liquid should be gone before continuing to the next step.

20. Take the plate off the magnet. Elute the RNA by adding 25-40 µL of nuclease free water. Pipette tip mix 10 times and incubate at room temperature for 5 minutes to complete elution.

21. Place the plate back on the magnet for 2 minutes, wait for the solution to turn completely clear. Transfer the clear RNA solution to a new plate or new tubes for storage (-20° C).

If beads are aspirated during the transfer, dispense the eluant back into the well and let the beads settle longer on the magnet to better compact the bead ring.

For Beckman Coulter's worldwide office locations and phone numbers, please visit www.beckmancoulter.com/contact

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