Agencourt RNAdvance Blood Kit

Supplemental Protocol for Micro RNA and Total RNA Isolation from PAXgene Preserved Blood

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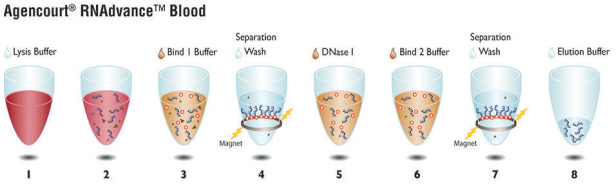
Process Overview



Introduction

The Agencourt RNAdvance Blood RNA purification kit utilizes Beckman Coulter's patented Agencourt SPRI paramagnetic bead-based technology to isolate total RNA from PAXgene preserved blood. The protocol is modified to isolate micro RNA (miRNA) and total RNA from 400 µL of PAXgene preserved blood per well in 96-well or 2 mL tube formats.

Note for miRNA and Total RNA Extraction

If the RNAdvance Blood kit is to be used for miRNA and total RNA isolation:

- **100% Isopropanol should not be added directly to the Wash Buffer bottle.** See *Reagent Preparation* for Wash Buffer preparation.
- 500 µL of Isopropanol is added in the Bind 1 at Step 4 instead of 400 µL (also see Step 3 of *Reagent Preparation*).
- Add 400 µL of Isopropanol in the Bind 2 Buffer at Step 16.
- 85% ethanol should be used instead of 70% ethanol in all ethanol washes.

General Remarks on Handling RNA

RNases are ubiquitous and general precautions should be followed in order to avoid their introduction to your 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.

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Materials Supplied by the User

Consumables and Hardware:

- 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)
- 1.7 mL microcentrifuge tubes (Fisher Scientific, NC9448938) for tube format
- 96-Well Riplate—2.2 mL (Ritter Medical, 43001-0200) for plate format
- 1.2 mL 96-well plate (ABGene #AB-1127; http://www.abgene.com)
- 37°C and 55°C water bath or heat block for proteinase K digestion and DNase treatment
- Plate Seals (ABGene #0580; http://www.abgene.com)

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)

Calculation of Yield

To determine yield of RNA, Beckman Coulter recommends using an OD260 measurement. To determine RNA quality, dilute samples to 1–2 ng/µL for analysis using the Agilent 2100 Bioanalyzer PicoChip assay.

Reagent Preparation

Prepare the following reagents in advance for both the 96-well and 2 mL tube protocols:

1. **Add PK Buffer to the Proteinase K tube/bottle. (Final concentration is 50 mg/mL.)**

   For the 50 prep kit, add 1.2 mL of PK Buffer per tube of Proteinase K.

   For the 384 prep kit, add 10 mL of PK Buffer to the bottle of Proteinase K.

   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.*

2. **Wash Buffer Preparation.**

   For miRNA and Total RNA Isolation—Add 100% Isopropanol to the Wash Buffer in a proportion of 1:1 (Isopropanol: Wash Buffer). Example: To make 10 mL of Wash Buffer solution, add 5 mL of 100% Isopropanol with 5 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 1:1.5 (Isopropanol: Wash Buffer). 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 seconds.

3. **Prepare Bind 1/Isopropanol Solution.**

   Prepare this Solution Fresh and Per Isolation—Discard Any Unused Solution. 510 µL of Bind 1/Isopropanol Solution are required per sample. Vortex the tube containing Bind 1 Buffer for at least 30 seconds to fully resuspend the beads. Combine 10 µL Bind 1 Buffer with 500 µL 100% Isopropanol and mix thoroughly.

4. **Prepare DNase Solution.**

   100 µL of 1X DNase solution are required per sample. Combine 80 µL nuclease-free water, 10 µL 10X DNase buffer, and 10 µL of DNase I. Make this solution fresh for each set of samples.

RNAdvance Blood miRNA Isolation Procedure

Thaw frozen tubes of PAXgene blood at room temperature. Cap the tubes tightly, then mix by inverting each tube several times or by vortexing.

1. **Aliquot 400 µL of PAXgene preserved blood into each well of a 2.2 mL processing plate or 1.7 mL tube.**

2. **Add Proteinase K and Lysis Buffer.**
   - Add 20 µL of Proteinase K (50 mg/mL, See *Reagent Preparation*, Step 1)
   - Add 300 µL of Lysis Buffer

   Mix thoroughly by pipetting up and down 10 times.

3. **Lysis and Protein Digestion.**

   Seal plate with a plate seal. Incubate samples in water bath at 55°C for 15 minutes. Before proceeding to the next step, let the samples cool for 2 minutes to room temperature.

   Note: *When using this plate in conjunction with a water bath, make sure the plate does not tip over and the seal does not get wet. Should the seal get wet or condensation form on the seal, spin the liquid down and very carefully remove the seal.*

4. **Shake Bind 1 Bottle vigorously to resuspend magnetic particles before using.**

   Prepare Bind 1/Isopropanol Solution as described in Reagent Preparation, *Step 3. Add 510 µL of Bind 1/ Isopropanol Solution to the samples and pipette mix 10 times. Incubate samples at room temperature for 5 minutes.*

5. **Place 2.2 mL processing plate on Agencourt SPRIPlate 96R-Ring Super Magnet Plate (or 1.7 mL tubes on SPRIStand) and separate for 15 minutes or wait for the solution to turn completely clear.**

6. **Fully remove supernatant from the 2.2 mL processing plate (or 1.7 mL tube) and discard.**

   This step must be performed while the 2.2 mL processing plate is situated on the magnet. *The following technique is recommended when working with opaque supernatant:* Place the pipette tip on the side of the well and carefully aspirate the liquid by following the liquid level down until approximately 200–250 µL remains in the well. Next, carefully place the pipette tip at the center of the bottom of the well, and slowly aspirate the remaining liquid, revealing the ring of beads.

7. **Remove the 2.2 mL processing plate (or 1.7 mL tube) from the magnet and wash the beads by adding 800 µL of Wash Buffer.**

   Pipette mix for 10 times. (Isopropanol must be added to Wash Buffer—See Reagent Preparation, *Step 2).* Pipette mix 10 times to resuspend the magnetic beads.

8. **Transfer the suspension to a 1.2 mL processing plate.**

   Be sure to transfer all of the sample solution and magnetic beads to the new plate (Skip this step if using tube format). Transferring the samples to the smaller plate allows for easier pipetting in subsequent steps.

9. **Place 1.2 mL processing plate (or 1.7 mL tube) on the magnet and separate for 7 minutes or wait for the solution to turn completely clear.**

10. **Completely remove supernatant from the 1.2 mL processing plate (or 1.7 mL tube) and discard.**

    This step must be performed while the plate is situated on the magnet. Do not to disturb the ring of separated magnetic beads.

11. **Remove the 1.2 mL processing plate (or 1.7 mL tube) from the magnet and add 800 µL of 85% ethanol.**

    Pipette mix 10 times to resuspend the magnetic beads.

12. **Return 1.2 mL processing plate (or 1.7 mL tube) to the magnet for 3 minutes or wait for the solution to turn completely clear.**

13. **Remove as much ethanol as possible and allow magnetic beads to dry for 3 minutes at room temperature.**

    Pipette slowly to avoid disturbing the beads. If too much ethanol is present (more than 5 µL), the DNase digestion will be inhibited, thereby affecting downstream applications.

14. **Remove the 1.2 mL processing plate from the magnet and add 100 µL of DNase solution**

    (See *Reagent Preparation*, Step 4).

    Pipette mix 5 times carefully—avoid bubbles and foaming.

15. **Seal plate with a plate seal and incubate 1.2 mL processing plate (or 1.7 mL tube) in water bath for 15 minutes at 37°C.**

16. **DO NOT REMOVE THE DNase SOLUTION.**

    Add 200 µL of Bind 2 Buffer plus 400 µL of 100% Isopropanol and pipette mix 10 times. Incubate at room temperature for 5 minutes.

    Note: *Do not mistake Bind 2 for Bind 1.*

17. **Place 1.2 mL processing plate (or 1.7 mL tube) onto the magnet for 5 minutes or wait for the solution to turn completely clear.**

18. **Remove supernatant and discard.**

    Wash by adding 800 µL of 85% ethanol. Pipette mix 10 times. Let sit for approximately 3 minutes and then remove ethanol while processing plate remains situated on the magnet plate.

19. **Repeat Step 18 one more time for a total of 2 ethanol washes.**

20. **Allow magnetic beads to dry for 3 to 5 minutes at room temperature.**

    Beads do not need to be completely dry, but the traces of liquid should be gone (i.e., droplets or puddles).

21. **Remove 1.2 mL processing plate (or 1.7 mL tube) from the magnet and elute RNA by adding 20 µL to 40 µL of nuclease-free water.**

    Pipette mix 10 times and incubate at room temperature for 2 minutes. On average, a 20 µL elution will produce a 20–50 ng/µL solution of RNA.

22. **Return the plate to the magnet for 2 minutes and carefully transfer eluted RNA away from the beads and into a fresh plate for storage.**

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For more information, visit www.Beckman.com or contact 1-800-369-0333. The RNAdvance Blood reagents are not intended or validated for use in the diagnosis of disease or other conditions.

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