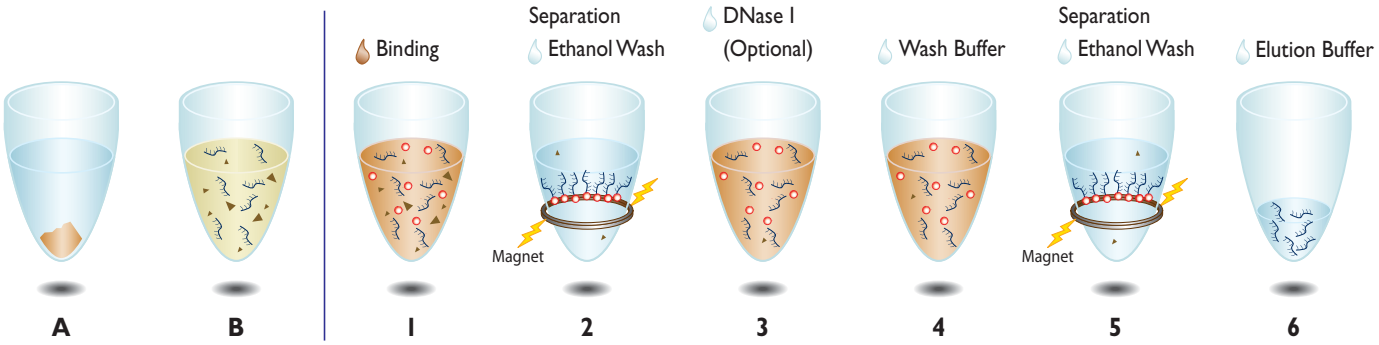
Agencourt® RNAdvance™ Tissue Kit

SUPPLEMENTAL PROTOCOL FOR Micro RNA AND TOTAL RNA ISOLATION FROM TISSUE

PROCESS OVERVIEW

Agencourt RNAdvance Tissue



Introduction

The Agencourt RNAdvance Tissue total RNA purification kit utilizes Beckman Coulter's patented Agencourt SPRI paramagnetic bead-based technology to isolate micro RNA (miRNA) and total RNA. The protocol can be performed in both 96-well and single tube formats. Purification begins with the homogenization and lysis of tissue. Following lysis, there is an immobilization of RNA onto the magnetic beads allowing for the RNA to be separated away from contaminants using a magnetic field. The RNA is then treated with DNase and the contaminants rinsed away using a simple wash procedure. The Agencourt RNAdvance Tissue kit is amenable to automation as it utilizes magnetic separation, thus eliminating the need for vacuum filtration or centrifugation.

Note for miRNA and Total RNA extraction

If the RNAdvance Tissue Kit is to be used for miRNA isolation:

- **100% Isopropanol should not be added directly to the Wash buffer bottle.** See page 3 for Wash buffer preparation
- 600 µL of Isopropanol is added in the Bind Buffer at step 9 instead of 400 µL
- 85% ethanol should be used instead of 70% ethanol in all ethanol washes

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)
- Tissue homogenizer
- 1.7 mL microcentrifuge tubes (Fisher Scientific, NC9448938) for tube format
- 96-Well Riplate-2.2 mL (World Wide Medical Products, 99181000) for plate format
- 37°C water bath or heat block for proteinase K digestion and DNase treatment

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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

Materials Supplied in the Kit

Reagent	Refered to in the Protocol As	Storage Conditions on Arrival	Storage Conditions once In Use (isopropanol, PK or buffer added)
Lysis Buffer	Lysis Buffer	Room Temperature	Room Temperature
Binding Buffer	Bind Buffer	4°C	4°C
Wash Buffer	Wash Buffer	Room Temperature	Room Temperature
Proteinase K	PK	-20°C	-20°C
Proteinase K Storage Buffer	PK Buffer	Room Temperature	-20°C

Kit Specifications

The Agencourt RNAdvance Tissue kit is manufactured under RNase-free conditions and has been tested and certified not to contain contaminating nucleases. The Agencourt RNAdvance Tissue kit can be used in 96 well and single tube formats.

General Remarks on Handling RNA

RNases are ubiquitous and general precautions should be followed in order to avoid the introduction of contaminating nucleases during the Agencourt RNAdvance Tissue 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.

Before starting

- Preheat water baths or heat blocks to 37°C
- Ensure that Agencourt RNAdvance Tissue Proteinase K and Wash Buffer have been assembled according to the following instructions:

Agencourt RNAdvance Tissue 96 Well and Tube Procedure

Assembly Steps 1 and 2 are only performed once for each new Agencourt RNAdvance Tissue kit. If these have already been completed during a previous experiment, please skip ahead to Step 3.

1. **Add PK Buffer to PK tube per the chart below:**

	50 Prep Kit Part # A32645	96 Prep Kit Part # A32649	384 Prep Kit Part # A32646
Volume of PK Buffer to Add	1.2 mL	2.3 mL	8.4 mL

Mix components by inverting the tube/ bottle several times. Do not vortex. Store this solution at -20°C when not in use.

2. **Add 100% isopropanol to Wash Buffer according to the instruction below.**

For miRNA and total RNA isolation: Add 100% Isopropanol to the Wash Buffer in a proportion of 2:1 (Isopropanol: Wash Buffer). 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.

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 to 6 mL of Wash Buffer in a 15 mL conical tube.

3. **Prepare Lysis Buffer**

Prepare this solution and use within 10 minutes – discard any unused solution.

For each sample combine 20 µL PK with 400 µL of Lysis Buffer. Example: for ten isolations mix 4.0 mL of Lysis with 200 µL of proteinase K. (It is generally recommended to prepare an additional 10% to account for dead volume.

Note: Pipette enzyme directly into the liquid and pipette mix up and down to remove any residual enzyme from the inside of the tip. A light vortex can be done to ensure homogeneity, but avoid foaming.

4. **Prepare Bind Buffer**

Prepare this solution fresh and per isolation – discard any unused solution. For each sample combine

80 µL Bind Buffer with 600 µL of isopropanol for a total of 680 µL.

5. **To perform the optional DNase step: Prepare DNase solution.**

Prepare this solution fresh and per isolation – discard any unused solution. Combine 80 µL nuclease

free water, 10 µL 10X DNase buffer, and 10 µL of DNase I.

6. **Homogenize up to 10 mg of tissue per 400 µL of Lysis Buffer.**

Note: Please refer to Appendix 1 of the RNAdvance Tissue Kit protocol for recommended homogenization methods and equipment (Agencourt RNAdvance Tissue protocol 000473v003, page 8-10).

Homogenization may be scaled up to any volume using 400 µL per 10mg tissue ratio. A larger lysate volume can be prepared and then split 400 µL lysate into each well or tube for extraction.
Complete homogenization and lysis of the tissue is a highly critical step in the isolation of high quality total RNA.

7. **Transfer 400 µL of homogenized lysate to processing plate (96-Well Riplate-2.2 mL), and seal with plate seal, or to a 1.7 mL microcentrifuge tube.**

8. **Incubate plate/tube in water bath for 25 minutes at 37°C.**

Following incubation lysate may be frozen indefinitely at -80°C.

9. **Shake Bind Buffer vigorously to resuspend magnetic particles before using. Add 680 µL of Bind Buffer and slowly pipette mix 10 times. Incubate at room temperature for 10 minutes.**

10. **Place on magnet for 10-20 minutes or wait until the solution turns completely clear (6-10 minutes for tube format).**

96 well plate format, use Agencourt SPRIPlate 96R Super Magnet Plate

Microcentrifuge tube format – use Agencourt SPRIStand.

11. **Fully remove supernatant from the plate/ tube and discard.**

    This step must be performed while the plate/ tube is situated on the magnet. Avoid disturbing the separated magnetic beads by aiming the pipette tip for the center of the well/ tube bottom. If beads are drawn out, leave a few microliters of supernatant behind. The liquid may be colored due to tissue homogenization.

12. **REMOVE plate/ tube from the magnet and wash by adding 1000 µL of Wash Buffer. Pipette mix 10 times.**

    Try to avoid bubbles while tip mixing.

13. **Return plate/ tube to the magnet for 5 minutes or wait for the solution to turn completely clear.**

14. **Fully remove supernatant from the plate/ tube and discard.**

    This step must be performed while the plate/ tube is situated on the magnet. Avoid disturbing the separated magnetic beads by aiming the pipette tip for the center of the well/ tube bottom. If beads are drawn out, leave a few microliters of supernatant behind. Colored liquid can be due to tissue homogenization.

15. **REMOVE plate/ tube from the magnet and wash by adding 800 µL of 85% ethanol per well and gently pipette mix 5 times.**

16. **Return plate/ tube to the magnet for 5 minutes or wait until the solution to turn completely clear.**

17. **Remove as much ethanol as possible, then, REMOVE the plate/ tube from the magnet.**

    Pipette slowly to avoid aspirating beads.

18. **Optional: Add 100 µL of DNase solution with the plate OFF the magnet. Incubate at room temperature for 1 minute without mixing to hydrate the beads.**

    If the DNase step is not required, skip to step 20. Perform only a total of 3 ethanol washes.

    Pipette mix 5 times to resuspend the beads in the DNase solution.

    Seal and incubate plate/ tube in a 37°C water bath for 15 minutes to facilitate digestion of DNA. DO NOT REMOVE THE DNase SOLUTION.

19. **Add 550 µL of Wash Buffer and pipette mix 5 times. Incubate at room temperature for 4 minutes.**

    Place plate/ tube onto the magnet and separate for 7 minutes or wait until the solution to turn completely clear

20. **Aspirate supernatant and wash by adding 600 µL of 85% Ethanol. Pipette mix 5 times.**

21. **Return plate/ tube to the magnet for 5 minutes or wait for the solution to turn completely clear.**

    Remove ethanol and discard.

22. **Repeat steps #20-21 for a total of 3 ethanol washes (including wash from #15 if omitting DNase step).**

    Remove final ethanol wash completely and allow beads to dry for 5 minutes at room temperature. (3 minutes at room temperature for tube format).

    Beads do not need to be completely dry, but all traces of liquid should be gone (i.e. no remaining droplets or puddles)

23. **Remove plate/ tube from the magnet and elute by adding a minimum of 40 µL of nuclease free water. Pipette mix 10 times and incubate at room temperature for 2 minute.**

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

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