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Agencourt RNAdvance Tissue Kit

Total RNA Isolation from Tissue

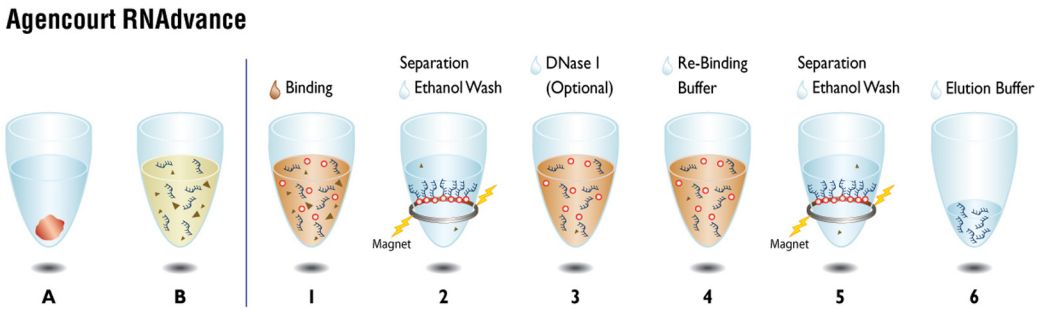
Please refer to www.beckmancoulter.com/ifu for updated protocols.

Agencourt RNAdvance Tissue: Table of Contents

Introduction

The Agencourt RNAdvance Tissue total RNA purification kit utilizes Beckman Coulter, Inc.'s patented Agencourt SPRI paramagnetic bead-based technology to isolate 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 particles allowing for the total 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. The following protocols are used for the isolation of total RNA from a maximum input of up to 10 mg of tissue in 400 µL of homogenization/lysis buffer per well in 96 well and 2 mL tube formats.

Process Overview



Manual Process

1. Add tissue to Lysis Buffer and mechanically homogenize
2. PK digestion

Automated or Manual Process

1. Add Bind Buffer
2. Separate magnetic beads from supernatant, and wash with Wash Buffer and Ethanol
3. DNase I (Optional)
4. Add Wash Buffer (Only required when optional DNase step is performed)
5. Separate magnetic beads from supernatant, and wash with Ethanol
6. Elute

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.

Working Under RNase Free Conditions

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 70% ethanol before starting work
- Treat electrophoresis gel boxes, including combs and gel trays, with 3% hydrogen peroxide for 10 minutes and rinse with DEPC treated water before use

Warnings and Precautions

DANGER	
Proteinase K: Proteinase K 80 – 100%	
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
P261	Avoid breathing vapours.
P280	Wear protective gloves, protective clothing and eye/face protection.
P284	In case of inadequate ventilation, wear respiratory protection.
P304+P340	IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

	**WARNING**
Rebinding Buffer: Guanidine Thiocyanate 10 – 20%	
H303	May be harmful if swallowed.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

	**WARNING**
Homogenization/Lysis Buffer: Guanidine Thiocyanate 20 – 30%	
Polyoxyethylated Octyl Phenol 1 – 2%.	
H303	May be harmful if swallowed.
H313	May be harmful in contact with skin.
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.
P273	Avoid release to the environment.
P280	Wear protective gloves, protective clothing and eye/face protection.
P312	Call a POISON CENTER or doctor/physician if you feel unwell.
P337+P313	If eye irritation persists: Get medical advice/attention.
SDS	Safety Data Sheet is available at *techdocs.beckmancoulter.com*.

Materials Supplied in the Kit

Reagent	Referred to in the Protocol as	Storage Conditions on Arrival	Storage Conditions once In Use (isopropanol, PK or buffer added)
Homogenization/Lysis Buffer	Lysis Buffer	Room Temperature	Room Temperature
Binding Buffer (magnetic component)	Bind Buffer	4°C	4°C
Wash- and Rebinding Buffer	Wash Buffer	Room Temperature	Room Temperature
Proteinase K	PK	-20°C	-20°C
Proteinase K Storage Buffer	PK Buffer	Room Temperature	-20°C

All components listed have a shelf-life of 6 months if properly stored.

Materials Supplied by the User

Consumables and Hardware

- **Agencourt SPRIPlate Magnetic Plate**

  For 96 well format: Beckman Coulter SPRIPlate 96R Ring Super Magnet Plate (Beckman Coulter product # A32782)

  For single tube format: Beckman Coulter SPRIStand (Tube kit only) (Beckman Coulter product # A29182)

  Reaction Plate:

  For 96 well format: For Prep Only: 1.2 mL 96 well sample processing plate (Thermo Scientific product # AB-1127)

  For single tube format: For Prep Only: 1.7 mL microcentrifuge tubes (or 1.5 mL microcentrifuge tubes, such as Thermo Fisher product # 05-408-129, or equivalent)

  Plate Seals (Thermo Scientific product # 0580)

- **Polypropylene Conical Centrifuge Tubes**, 15 ml or 50 ml (BD Falcon product # 352097 (15 ml) or 352098 (50 ml)) for homogenization (recommended, not required)
- **37°C water bath**
- **Liquid handling robotics or a multi-channel hand pipette**

Reagents

- **100% Isopropanol** (American Bioanalytical product # AB-07015)
- **70% Ethanol made with nuclease free water** (American Bioanalytical product # AB-00138)

  NOTE 70% Ethanol is hygroscopic, prepare fresh 70% Ethanol regularly for optimal results.

- **Optional: DNase I (RNase-free)** [(2 U/µL); Ambion Inc. product # 2222]
- **Optional: DNase I 10X buffer** (Ambion Inc. product # 8170G)
- **Reagent grade water, nuclease-free** (Ambion product # 9932)

Calculation of Yield

To gauge yield of RNA, Beckman Coulter recommends using a 260/280 reading or visualization on agarose gel. These methods will give the most accurate quantitation of RNA.

Procedure

Agencourt RNAdvance Tissue 96 Well Plate and Tube Protocol

Assembly Steps 1 and 2 are only performed once for each new Agencourt RNAdvance Tissue kit. If you have already made the following preparations previously, please skip ahead to Step 3.

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

	50 Prep Kit Part #A32645	**96 Prep Kit Part#A32649**	**384 Prep Kit Part #A32646**
Volume of Isopropanol to add	40 mL	62 mL	250 mL

Mix thoroughly.

3. **Prepare Lysis Buffer**

   NOTE Prepare this solution and use within **10 minutes**. Discard any unused solution.

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

   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**

   NOTE Prepare this solution fresh and per isolation – discard any unused solution.

   For each sample combine 80 µl Bind Buffer with 320 µl of isopropanol for a total of 400 µl.

5. **If you wish to perform the *optional* DNase step:**
   a. Prepare DNase solution

      NOTE Prepare this solution fresh and per isolation - discard any unused solution.

   b. Combine 80 µL nuclease free water, 10 µL 10 x 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 - Recommended Homogenization Methods* for recommended homogenization methods and equipment.

   Homogenization may be scaled up to any volume using this ratio.

   NOTE Only 400 µL of lysate may be processed at a time.

   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 the homogenized lysate to processing plate (AB-1127) and seal with plate seal. Or transfer to a 1.7 ml microcentrifuge tube.**
8. **Incubate the plate/ tube in a water bath for 25 minutes at 37°C.**

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

9. **Shake the Bind Buffer to resuspend magnetic particles before using. Add 400 µL of Bind Buffer and slowly pipette mix 5 times. Incubate at room temperature for 5 minutes.**

   Try to avoid bubbles while tip mixing. Some bead clumping may occur; it will not affect the quality or yield.

10. **Place on magnet for 6 minutes. For 96 well plates - Agencourt SPRIPlate 96R Super Magnet Plate or for microcentrifuge tube format - Beckman Coulter SPRIStand.**

    Wait for the solution to clear before proceeding to the next step.

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 or tube bottom. If beads are drawn out, leave a few microliters of supernatant behind. Colored liquid can be due to tissue homogenization.

12. **Remove the plate/ tube from the magnet and wash by adding 800 µL of Wash Buffer. Pipette mix 10 times.**

    Try to avoid bubbles while tip mixing.

13. **Return plate or tube to the magnet for 5 minutes.**

    Wait for the solution to clear before proceeding to the next step.

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

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

15. **Remove the plate or tube from the magnet and wash by adding 800 µL of 70% ethanol per well and gently pipette mix 4 times.**

    Beads do not have to be fully resuspended in this step.

16. **Return plate/ tube to the magnet for 5 minutes.**

    Wait for the solution to clear before proceeding to the next step.

17. **Remove as much ethanol as possible, then remove the plate or tube from the magnet.**

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

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 you do not wish to perform the DNase step, skip to step 23. Perform only a total of 3 ethanol washes.

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

20. **Seal and incubate plate/ tube in a 37°C water bath for 15 minutes to facilitate digestion of DNA.**

21. ***DO NOT REMOVE THE DNase SOLUTION.*** **Add 550 µL of Wash Buffer and pipette mix 5 times. Incubate at room temperature for 4 minutes.**

22. **Place plate/ tube onto the magnet and separate for 7 minutes.**

    Wait for the solution to clear before proceeding to the next step.

23. **Aspirate supernatant and wash by adding 600 µL of 70% Ethanol. Do not pipette mix.**

    This step is performed on the magnet.

24. **Incubate for 2 minutes to allow beads to resettle. Remove ethanol and discard.**

25. **Repeat steps #23 and #24 for a total of 3 ethanol washes (including wash from #15 if omitting DNase step).**

26. **Remove final ethanol wash completely and allow beads to dry for 10 minutes at room temperature.**

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

27. **Remove plate or 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 minutes.**

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

Appendix 1 - Recommended Homogenization Methods

Complete homogenization of the tissue is a critical step in isolating high quality total RNA. Incomplete homogenization may result in lower yields and decreased purity of the isolated RNA. Several protocols for the homogenization of various tissues are commercially available. Selection of the best method for a particular range of uses should be determined experimentally. The homogenization protocols and equipment listed below have been used successfully in our laboratory.

- Keep tissue frozen on dry ice as much as possible to minimize degradation.
- Keep plastics, forceps, and cutting tools on dry ice to minimize degradation.
- RNAdvance Tissue Lysis Buffer contains RNAse inhibitors. RNA will begin to degrade when thawed if it is not in contact with Lysis Buffer. Therefore place the frozen tissue directly into the Lysis Buffer before it begins to thaw. Homogenize as quickly as possible to stabilize the entire piece of tissue.
- Weigh tube without tissue and re-weigh with tissue to make sure that the appropriate amount of Lysis Buffer and PK are added to the sample.
- If foaming is a problem in a 1.7 mL tube, then homogenization in 15 or 50 mL tubes is recommended.
- If you have enough starting material, make up an extra sample to compensate for dead volume and any foaming that may occur during and post homogenization. For example if processing two tissues samples (20 mg) homogenize enough for 3 (30 mg).
- If using a rotor stator homogenizer, homogenize using an up and down motion in the tube. Avoid heating up the sample because this can degrade the RNA.

Recommended Equipment for Homogenization

For tube format: tissue dispersing device: IKA Ultra Turrax using a 5 mm dispersing element or Brinkman Polytron homogenizer

For 96 well plate format: Plate vortexer (Troemner VX2400 Multitube Vortexer) and two 3.2 mm stainless steel beads (part number 11079132ss) per well. 2 ml deepwell plate (Product # AB-0661); plate seal (Product # AB-0580).

In general tissues may be divided into three groups or types for the purpose of homogenization:

1. soft tissues such as liver, kidney, and lung
2. fibrous tissues, such as skeletal, cardiac and vascular smooth muscle
3. lipid rich tissues, such as adipose and brain.

(1) Tube Format:

Up to 10 mg of tissue may be homogenized in 400 µL of Lysis Buffer/ PK in a 1.7 µl microcentrifuge tube. Larger amounts of tissue may be homogenized simply by using a 15ml/ 50ml conical tube and scaling up the volume of the homogenization. Use an additional 400 µl of Lysis/PK for ever additional 10mg of tissue. For volumes above 2 mL total, a larger tissue dispersing element should be used (8 to 10 mm) to ensure complete homogenization. Foaming of the sample during lysis can be minimized by keeping the dispersing element in a fixed location in the center of the tube and as close to the bottom as possible.

Soft tissue, tube format: Samples should be homogenized at the highest speed setting for about 2 minutes.

Fibrous tissue, tube format: Samples should be homogenized at the highest speed setting for about 5 minutes. In addition the 37°C lysis incubation can be extended to 45 minutes for particularly tough tissue such as vascular smooth muscle.

Lipid Rich Tissues, tube format: Samples should be homogenized at the highest speed setting for about 30 – 90 seconds. Special care should be taken to avoid excessive foaming of lipid rich tissues during homogenization.

(2) 96 Well Plate Format:

Homogenization of up to 10 mg of tissue in 400 µL of Lysis Buffer/ PK may be accomplished using either a tissue dispersing device or metal beads and agitation ("bead beating"). The bead beating method provides a higher throughput solution for 96 well plate isolations. Add 400 µL of Lysis Buffer per well and up to 10 mg of tissue. Seal the plate with a plastic plate seal and shake vigorously (2400 RPM) in a plate vortexer for homogenization. Incubate the deep well plate at 37°C without removing the metal beads. Upon completion of the incubation the lysate should be transferred to a 1.2 mL plate for processing.

Soft tissue, plate format: Samples should be homogenized about 10 minutes at 2400 RPM.

Fibrous tissue, plate format: vortex about 20 to 25 minutes at 2400 RPM.

NOTE Some fibrous tissue may not be completely dispersed using metal beads – specific homogenization requirements for some fibrous tissues may need to be determined experimentally.

Lipid Rich Tissues, plate format: vortex for about 10 minutes at 2400 RPM.

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