

Agencourt® RNAdvance® Cell v2

Total RNA Isolation from Cultured Cells

Please refer to <http://www.agencourt.com/technical> for updated protocols and refer to MSDS instructions <http://www.beckmancoulter.com/customer-support/msds/msds.asp> when handling or shipping any chemical hazards.

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Introduction:

The Agencourt RNAdvance Cell v2 Total RNA extraction kit utilizes Agencourt's patented SPRI® paramagnetic bead-based technology to isolate total RNA from 200 to 50,000 cultured eukaryotic cells (cell lines or primary cells). The protocol is designed for high-throughput processing of 96 well plates and is easily amenable to automation as it uses magnetic separation instead of vacuum filtration or centrifugation.

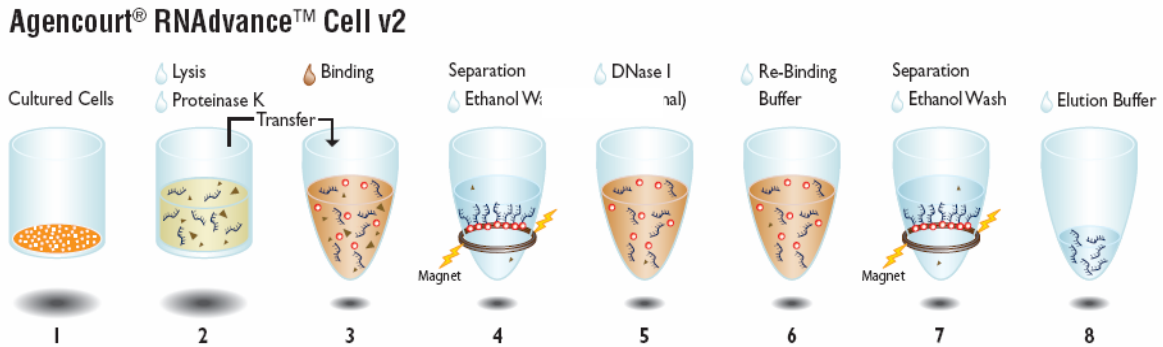
During the RNAdvance Cell v2 process, a solution of Proteinase K and Lysis Buffer is used to break open cells, digest proteins and inactivate RNases. A magnetic Bind Buffer is then added to the lysate, causing Total RNA to be immobilized onto magnetic particles. This differential binding allows Total RNA to be quickly separated from contaminants using a magnetic field. Once bound to the magnetic beads, the RNA can be treated with DNase and the contaminants rinsed away using a simple washing procedure. The purified RNA is eluted using nuclease-free water.

RNAdvance Cell v2 does not extract microRNAs.



Innovate Automate
SIMPLIFY

Process Overview:



1. Start with cultured cells or primary cells.
2. Lyse cultured cells with Lysis Buffer and Proteinase K, transfer into new plate.
3. Bind Total RNA to paramagnetic beads
4. Separate beads from contaminants, wash with Wash Buffer & Ethanol
5. Add DNase to digest genomic DNA
6. Re-bind RNA to beads with Wash Buffer & remove contaminants
7. Wash the magnetic beads with 70% Ethanol to remove residual contaminants
8. Elute RNA from magnetic particles.

Materials Supplied in the Kit:

The Agencourt RNAdvance Cell v2 kit is manufactured under RNase-free conditions and has been tested and certified not to contain contaminating nucleases. The reagents have a shelf life of 6 months if stored as directed:

Reagent	Referred to in the Protocol As	Storage Conditions on Arrival	Storage Conditions once In Use (isopropanol or buffer added)
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	Room Temperature

For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186

Materials Supplied by the User:**Consumables and Hardware:**

- **Magnetic Separator:** Agencourt SPRIPlate 96 Ring Super Magnet plate; Agencourt #000322; Beckman #A32782
<http://www.agencourt.com/>
- **Culture Plate:** Costar 9017: 300ul Flat Bottom Culture Plate; Fisher Scientific #07-200-98
- **Prep Plate:** Magnet compatible 96 well plate:
(manual) 1.2mL ABGene #AB-1127; <http://www.abgene.com>
(manual) Costar 3797: 300uL Round Bottom Culture Plate; Fisher Scientific 07-200-105.

Reagents:

- **100% Isopropanol;** American Bioanalytical # AB-07015; <http://www.americanbio.com/>
- **70% Ethanol made with nuclease free water** (*Note: 70% Ethanol is hygroscopic, prepare fresh 70% Ethanol regularly for optimal results*) American Bioanalytical # AB-00138; <http://www.americanbio.com/>
- **DNase I (RNase-free) & DNase I Buffer** (2 U/ μ L); Ambion #AM2224 (contains both components), <http://www.ambion.com>
 - **OR DNase I (RNase-free)** (2 U/ μ L); Ambion #AM2222 **AND DNase I 10X Buffer** Ambion #AM8107G, <http://www.ambion.com>
- **Reagent grade water, nuclease-free** (Ambion #AM9932; <http://www.ambion.com>)

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 Cell v2 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

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- 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

Procedure:

Agencourt RNAdvance Cell v2 was designed for routine extraction of RNA from 200 to 50,000 cells¹ per prep. The Agencourt RNAdvance **Tissue** kit is recommended for 50,000 – 2 million cells per prep.

1. For each **new** kit, assemble Proteinase K and Wash Buffer **once**. Mark each tube or bottle with the date of assembly:

	Proteinase K Solution (50 mg/mL)	Wash Buffer
	Volume of PK Buffer to add to lyophilized Proteinase K	Volume of 100% Isopropanol to add to Wash Buffer
96 Prep kit #001354/ A47942	400 µL	16 mL
960 Prep kit #001355/ A47943	4 mL	160 mL
Storage Condition	-20°C	Room Temperature

¹ For some cell lines, up to 100,000 cells may be used with Agencourt RNAdvance Cell v2. Cell lines that are easy to lyse and have low amounts of genomic DNA may be more successful than others.

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2. **Preparation of Solutions** – Make these reagents up fresh every time. Discard any unused solution after use. It is generally recommended to prepare an additional 10% to account for pipetting error.
 - **Prepare fresh 70% Ethanol**
 - **Prepare Bind Buffer Solution** – Shake or vortex Bind Buffer to resuspend the magnetic particles. For each sample, combine 80µL Bind Buffer with 95 µL of Isopropanol for a total of 175 µL Bind Buffer Solution.
 - **Prepare DNase Solution** – For each sample, combine 20 µl DiH₂O, 2.5 µL 10X DNase I buffer and 2.5 µL DNase I, for a total of 25 µl DNase Solution.
 - **Prepare Lysis/PK Solution** – **USE WITHIN 30 MINUTES** – For each 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.

3. **Remove the culture medium from the cells as completely as possible by pipetting.**
 For cell-culture plates, tip the plate slightly to one side and place the pipette tip in the corner of the well when aspirating. For cells grown in suspension, first pellet cells then carefully remove media.

4. **Add 63 µL of Lysis/PK Solution (prepared in Step 2) to each sample. Gently pipette tip mix 20 times at the bottom of the well to resuspend the cells.**

5. **Incubate the samples for 30 minutes at room temperature to complete the lysis and digestion.**
 Possible Stop Point: Once the 30 minute incubation is complete, the lysate can be frozen at -80°C and extracted at a later time. If freezing samples, seal the plate with an adhesive seal to prevent contamination. Thaw samples at room temperature before resuming the Agencourt RNAdvance Cell v2 process.

6. **Transfer the entire lysate from the sample plate into a magnet-compatible 96 well round bottom plate. For manual processing, Abgene AB-1127 or Costar 3797 Fisher Scientific 07-200-105 plates are recommended.**

7. **Shake or mix Bind Buffer Solution (prepared in Step 2) to resuspend magnetic particles. Add 175 µL of Bind Buffer Solution to each sample and pipette tip mix 10 times or until homogeneous.**
 During this step, nucleic acids bind to the magnetic particles. Isopropanol may float to the top of the liquid column so it is important to mix very well to incorporate the Bind Buffer

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Solution. For best results, use a mix volume that is slightly less than the total volume in the well.

8. **Incubate the samples for 5 minutes at room temperature to bind.**
9. **Place the sample plate on an Agencourt SPRIPlate 96 Ring Super Magnet for 5 minutes or until the solution clears. 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.
10. **Take the plate off the magnet. Add 200 μ L of Wash Buffer. Pipette tip mix 10 times, or until the magnetic particles are fully resuspended.**
It is normal for a few beads clumps to remain after resuspension.
11. **Place the plate back on the magnet for 5 minutes, or until the solution clears. 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.
12. **Take the plate off the magnet. Add 200 μ L of 70% Ethanol. Gently pipette tip mix 5 times, or until beads are fully resuspended.**
13. **Place the plate back on the magnet for 5 minutes, or until the solution clears. Thoroughly remove and discard as much of the ethanol wash as possible.**
Excess ethanol can reduce the activity of DNase during the next steps.
14. **Take the plate off the magnet. Add 25 μ L of DNase Solution (prepared in Step 2) 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.
15. **Incubate the sample plate at room temperature for 15 minutes to complete the DNase digestion.**
16. **DO NOT REMOVE THE DNase SOLUTION. Add 138 μ L 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.

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17. Incubate the plate at room temperature for 5 minutes to bind.
18. Place the plate on the magnet for 5 minutes or until the solution clears. Remove and discard the supernatant.
19. **Take the plate off the magnet.** Wash the beads by adding 200 μ L of 70% ethanol. Pipette tip mix 5 times, or until beads are fully resuspended. Ethanol washes remove salt, Wash Buffer and any residual contaminants.
20. Place the sample plate on the magnet for 5 minutes or until solution clears. Remove ethanol and discard.
21. Repeat steps 19-20 one more time for a total of 2 ethanol washes.
22. Remove as much of the final ethanol wash as possible. Allow the beads to dry for 10 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.
23. **Take the plate off the magnet.** Elute the RNA by adding 40 μ L of nuclease free water. Pipette tip mix 10 times and incubate at room temperature for 5 minutes to complete elution.
24. Place the plate back on the magnet for 2 minutes, or until the solution clears. 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 plate sit longer to better compact the bead ring. Leave 5 μ L of eluant behind to avoid bead carry-over. During the transfer, place the pipette tip in the center of the bead ring and aspirate slowly.

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