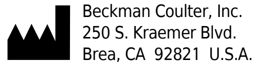
Instructions For Use

RNAdvance Cell v2

Total RNA Isolation from Cultured Cells

B66718AD
June 2019



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Contact Us

- For questions regarding this protocol, call Technical Support at Beckman Coulter at 1-800-369-0333.
- For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-742-2345 (USA or Canada) or contact your local Beckman Coulter Representative.
- Refer to *www.beckman.com/techdocs* for updated protocols.

Glossary of Symbols is available at www.beckman.com/techdocs (PN C05838).

Product Availability

REF A47942 — RNAdvance Cell v2, 96 Prep Kit

REF A47943 — RNAdvance Cell v2, 960 Prep Kit

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Revision History

This document applies to the latest version and higher versions. When a subsequent version changes the information in this document, a new issue will be released to the Beckman Coulter website. For updates, go to www.beckman.com/techdocs and download the latest version of the manual.

Revision AC, 05/2019
Updates include: Format and content updates throughout the manual.

Revision AD, 06/2019
Updates include: *Sample Preparation*

Protocol for Total RNA Isolation from Cultured Cells

RNAdvance Cell v2 *is for molecular biology research use only. Not for use in diagnostic procedures.*

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Product Description

The RNAdvance Cell v2 Total RNA extraction kit utilizes Beckman Coulter Inc.'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 microRNA.

Kit Specifications

Kit Part Number	Number of Preps
A47942	96 preps
A47943	960 preps

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 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
- 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

Statement of Warnings

	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.
	P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
	P403+P233	Store in a well-ventilated place. Keep container tightly closed.
	SDS	Safety Data Sheet is available at *www.beckman.com/techdocs*.

	DANGER	
	Wash WBD: Guanidine Thiocyanate 10 – 20%	
	H303	May be harmful if swallowed.
	P314	Get medical advice/attention if you feel unwell.
	SDS	Safety Data Sheet is available at *www.beckman.com/techdocs*.

	DANGER	
	Lysis LBE: Guanidine Thiocyanate 20 – 30%	
	Polyoxyethylated Octyl Phenol <1%	
	H303	May be harmful if swallowed.
	H313	May be harmful in contact with skin.
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
	P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
	P363	Wash contaminated clothing before reuse.
	P405	Store locked up.
	P501	Dispose of contents/container in accordance with local/national regulations.
	SDS	Safety Data Sheet is available at *www.beckman.com/techdocs*.

Storage and Stability

NOTE Refer to the product labels for expiration dates.

Reagent	Storage Condition
Lysis LBE	**Room Temperature**
Bind BBC	**4°C**
Wash WBD	**Room Temperature**
Proteinase K	**-20°C**
Proteinase K Buffer	**Room Temperature**

Materials Supplied

Reagent	96 Preps Kit (A47942)	960 Preps Kit (A47943)	Symbol
Lysis LBE	REF C39465	REF C42082	LBE
Bind BBC	REF C42084	REF C42086	BBC
Wash WBD	REF C39486	REF C39489	WBD
Proteinase K	REF C42092	REF C42093	–
Proteinase K Buffer	REF C42302	REF C42302	–

Materials Required but not Supplied

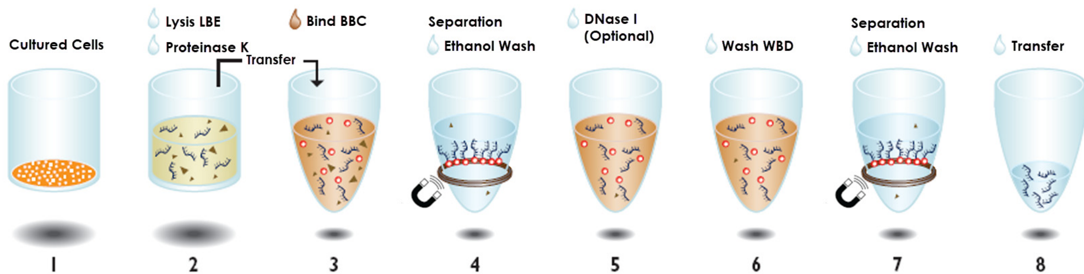
Consumables and Hardware

Item	Type
Magnetic Separator	**SPRIPlate 96R - Ring Super Magnet Plate** (Beckman Coulter product # A32782, *www.beckman.com*)
Culture Plate	**Costar 9017: 300 µL Flat Bottom Culture Plate** (Fisher Scientific product # 07-200-98)
Prep Plate	**96 well 1.2 mL** (Thermo Scientific product # AB-1127)
	Costar 3797: 300 µL Flat Bottom Culture Plate (Fisher Scientific product # 07-200-105)

Reagents

Item	Supplier & Catalog Number
100% Isopropanol	American Bioanalytical # AB-07015
70% Ethanol, Made With **Nuclease-Free Water** **NOTE** **70% Ethanol** is hygroscopic. Prepare fresh **70% Ethanol** regularly for optimal results.	American Bioanalytical # AB-00138, ***or equivalent***
DNase I (RNase-Free) & **DNase I Buffer** (2 U/µL) ***OR*** **DNase I** (RNase-free) (2 U/µL) **DNase I 10X Buffer**	Ambion product # AM2222 (contains both components) ***OR*** Ambion product # AM2222 Ambion product # AM8107G
Reagent Grade Water, Nuclease-Free	Ambion product # AM9932, ***or equivalent***

Process Overview



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

Sample Preparation

RNAdvance Cell v2 was designed for routine extraction of RNA from 200 to 50,000 cells* per prep.

NOTE The RNAdvance ***Tissue*** kit is recommended for **50,000 – 2 million cells per prep**.

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

	Proteinase K Solution (50 mg/mL)	Wash WBD
	Volume of PK Buffer to add to lyophilized Proteinase K	Volume of 100% Isopropanol to add to Wash WBD
96 Prep kit A47942	400 µL	62 mL
960 Prep kit A47943	4 mL	250 mL
Storage Condition	-20°C	Room Temperature

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** BBC solution – Shake or vortex **Bind** BBC to resuspend the magnetic particles. For each sample, combine **80 µL** of **Bind** BBC with **95 µL** of **Isopropanol** for a total of **175 µL Bind** BBC solution
- Prepare **DNase Solution** – For each sample, combine **20 µL** of **DiH_2O**, **2.5 µL** of **10X DNase I** buffer and **2.5 µL** of **DNase I**, for a total of **25 µL** of **DNase Solution**.
- Prepare **Lysis** LBE **/Proteinase K Solution** – ***USE WITHIN 30 MINUTES*** – For each sample combine **3 µL** of **Proteinase K** (50 mg/mL) with **60 µL** of **Lysis** LBE, for a total of **63 µL** of **Lysis** LBE **/Proteinase K Solution**. Mix gently to avoid creating bubbles.

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

Procedure

1 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.

2 Add **63 µL** of **Lysis** LBE **/Proteinase K Solution** (prepared in *Sample Preparation*, step 2) to each sample. Gently pipette tip mix **20 times** at the bottom of the well to resuspend the cells.

3 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 RNAdvance Cell v2 process.

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

5 Shake or mix **Bind** BBC **Solution** (prepared in *Sample Preparation*, step 2) to resuspend magnetic particles. Add **175 µL** of **Bind** BBC **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** BBC **Solution.** For best results, use a mix volume that is slightly less than the total volume in the well.

6 Incubate the samples for **5 minutes** at **room temperature** to bind.

7 Place the sample plate on a SPRIPlate 96R - Ring Super Magnet Plate 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.

8 ***Take the plate off the magnet.*** Add **200 µL** of **Wash** WBD. 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.

9 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.

10 ***Take the plate off the magnet.*** Add **200 µL** of **70% Ethanol**. Gently pipette tip mix **5 times**, or until beads are fully resuspended.

11 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.

12 ***Take the plate off the magnet.*** Add **25 µL** of **DNase Solution** (prepared in *Sample Preparation*, 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.

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

14 ***DO NOT REMOVE THE DNase SOLUTION.*** Add **138 µL** of **Wash** WBD to each sample and pipette tip mix **10 times**, or until homogeneous.

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

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

16 Place the plate on the magnet for **5 minutes** or until the solution clears. Remove and discard the supernatant.

17 ***Take the plate off the magnet.*** Wash the beads by adding **200 µL** of **70% Ethanol**. Pipette tip mix **5 times**, or until beads are fully resuspended.

Ethanol washes remove salt, **Wash** WBD and any residual contaminants.

18 Place the sample plate on the magnet for **5 minutes** or until solution clears. Remove **Ethanol** and discard.

19 Repeat steps 17-18 one more time for a total of two **Ethanol** washes.

20 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.

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

22 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 µL** 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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