Instructions For Use

RNAdvance Viral XP

RNA Isolation
from Viral Transport Media

PN C58637AB
May 2020

Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821 U.S.A.

BECKMAN COULTER

RNAdvance Viral XP
RNA Isolation from Viral Transport Media
Instructions for Use
PN C58637AB (May2020)

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 C59543 — RNAdvance Viral XP

Find us on the World Wide Web at:
www.beckman.com

Beckman Coulter Eurocenter S.A.
22, rue Juste-Olivier
Case Postale 1044
CH - 1260 Nyon 1, Switzerland
Tel: +41 (0) 22 365 36 11

May be covered by one or more pat. - see www.beckman.com/patents

Original Instructions

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.

Initial Issue, C58637AA, 04/2020

Revision AB, 05/2020
Updates were made to the following section: *Materials Supplied*.

Note: *Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.*

Protocol for RNA Isolation from Viral Transport Media

RNAdvance Viral XP *is for research use only. Not for use in diagnostic procedures.*

Table of Contents

Product Description

The RNAdvance Viral XP RNA purification kit utilizes Beckman Coulter, Inc.'s SPRI paramagnetic bead-based technology to isolate RNA from viral transport media. The protocol can be performed in both 96-well plate and single tube formats. Purification begins with lysis. Following lysis, the nucleic acid is immobilized onto the magnetic particles, allowing separation from contaminants using a magnetic field. The contaminants are rinsed away using a simple wash procedure, and the nucleic acid is eluted in nuclease free water. The RNAdvance Viral XP 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 RNA from 200 µL of viral transport media per well in 96-well plate and 1.5 mL tube formats.

Kit Specifications

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

Kit Part Number	Number of Preps
C59543	1,056 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 Viral XP 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

- Use Good Laboratory Practices (GLP) and follow laboratory, local, or national safety guidelines when handling any virus or reagent.
- Specimens, samples, and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
- Do not mix reagents or waste from this kit with bleach, harmful gases may be released.
- Do not use reagents beyond the expiration date on the vial label.

DANGER	
Lysis LBF: Boric Acid 0.1 – 1%	
Tris(hydroxymethyl)-aminomethane 1 – 3%	
Polyoxyethylated Octyl Phenol 1 – 2%	
Guanidine Thiocyanate 30 – 40%	
H302	Harmful if swallowed.
H313	May be harmful in contact with skin.
H316	Causes mild skin irritation.
H319	Causes serious eye irritation.
H360	May damage fertility or the unborn child.
H412	Harmful to aquatic life with long lasting effects.
P201	Obtain special instructions before use.
P273	Avoid release to the environment.
P280	Wear protective gloves, protective clothing and eye/face protection.
P308+P313	If exposed or concerned: Get medical advice/attention.
P332+P313	If skin irritation occurs: Get medical advice/ attention.
P337+P313	If eye irritation persists: Get medical advice/attention.
SDS	Safety Data Sheet is available at *www.beckman.com/techdocs*.

NOTE

Lysis LBF SDS part number: C43723
Bind VBE SDS part number: C58717.

Storage and Stability

NOTE Refer to the product labels for expiration dates. Use within 30 days of opening.

Reagent	Storage Condition
Lysis LBF	**15-30°C**
Bind VBE	**15-30°C**

Materials Supplied

Reagent	1056 Preps Kit (C59543)	Symbol
Lysis LBF	REF **C42153**	
Bind VBE	REF **C58702**	

Materials Required but not Supplied

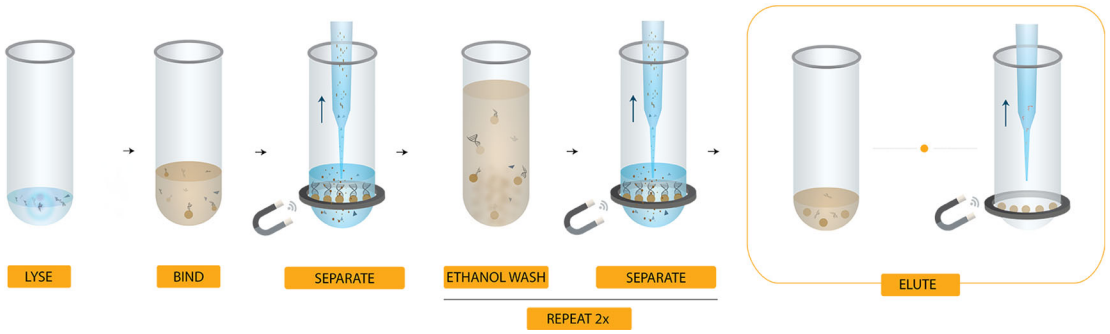
Consumables and Hardware

Format	Item	Type
96-Well Plate	**Magnetic Separator**	**SPRIPlate 96R - Ring Super Magnet Plate** (Beckman Coulter product # A32782, *www.beckman.com*)
	Reaction Plate	**1.2 mL 96-Well Plate** (Thermo Fisher product # AB-1127)
	Plate Seals	Thermo Fisher product # 0580, ***or equivalent***
Tube	**Magnetic Separator**	**SPRIStand Magnetic 6 Tube Stand** (Beckman Coulter product #A29182, *www.beckman.com*)
	Tube	**1.5 mL Microcentrifuge Tubes** (Eppendorf product #022431021 ***or equivalent***)

Reagents

Item	Supplier & Catalog Number
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***
Reagent Grade Water, Nuclease-Free	Ambion product # AM9932, ***or equivalent***

Process Overview



1. Add **Lysis** (LBF) to **viral transport media** and mix.
2. Addition of **Bind** (VBE)
3. Magnetic separation of beads from supernatant, wash with **Ethanol**.
4. Elution.

Procedure

Part A — RNAdvance Viral XP 96-Well Plate Protocol

Swabs should be stored per swab manufacturer's instructions. If swab includes transport media, sample should be mixed per manufacturer's instructions and the transport media should be used for the extraction. If the swab does not include transport media, a commercial or lab-made transport media can be used. PBS can also be used in place of transport media. All samples should be well mixed before extraction. Beckman Coulter recommends RNA be checked for yield and purity for downstream applications. We also recommend the user spike samples with control RNA to validate downstream assays.

1 Prepare sample.

- Vortex the viral transport media or PBS for **10 seconds** at maximum speed to resuspend the sample.
- Briefly pulse the media in a centrifuge to collect liquid on the tube cap.
- Aliquot **200 µL** of sample into each well of a 1.2 mL processing plate.

2 Add **150 µL** of **Lysis** (LBF)

Mix thoroughly by pipetting up and down **10 times**.

3 Lysis:

Seal plate with a plate seal. Incubate samples at **room temperature** (15-30°C) for **20 minutes**.

4 Mix the bottle of **Bind** (VBE) to fully resuspend the beads.

5 Add **350 µL** of **Bind** (VBE) to the samples and pipette mix **10 times** or until well mixed. Incubate samples at **room temperature** (15-30°C) for **5 minutes**.

6 Place 1.2 mL processing plate on SPRIPlate 96R Super Magnet Plate and separate for **10 minutes**.

7 Fully remove supernatant from the 1.2 mL processing plate and discard.

This step must be performed while the 1.2 mL processing plate is situated on the magnet.

8 Remove the 1.2 mL plate from the magnet and wash by adding **400 µL** of **70% Ethanol** to the sample. Pipette mix 10 times, or until well mixed.

9 Place 1.2 mL processing plate on the magnet and separate for **2 minutes**. Wait for the solution to clear before proceeding to next step.

10 Completely remove supernatant from the 1.2 mL processing plate and discard. This step must be performed while the plate is situated on the magnet. Do not disturb the ring of separated magnetic beads.

11 Repeat steps 8 - 10 for a total of 2 washes.

12 Allow magnetic beads to dry for **1 minute** at **room temperature** (15-30°C).

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

13 Remove 1.2 mL processing plate from the magnet and elute nucleic acid by adding **40 µL** of **Nuclease-Free Water.** Pipette mix **10 times** and incubate at **room temperature** (15-30°C) for **5 minutes.**

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

NOTE Beckman Coulter recommends RNA samples be stored on ice for immediate use, or stored at -20 to -80°C for long-term storage.

Part B — RNAdvance Viral XP 1.5 mL Tube Protocol

Swabs should be stored per swab manufacturer's instructions. If swab includes transport media, sample should be mixed per manufacturer's instructions and the transport media should be used for the extraction. If the swab does not include transport media, a commercial or lab-made transport media can be used. PBS can also be used in place of transport media. All samples should be well mixed before extraction. Beckman Coulter recommends RNA be checked for yield and purity for downstream applications. We also recommend the user spike samples with control RNA to validate downstream assays.

1 Prepare sample.

- Vortex the viral transport media or PBS for **10 seconds** at maximum speed to resuspend the sample.
- Briefly pulse the media in a centrifuge to collect liquid on the tube cap.
- Aliquot **200 µL** of **viral transport media** or **PBS** into each well of a 1.5 mL microcentrifuge tube.

2 Add **150 µL** of **Lysis** LBF

Mix thoroughly by pipetting up and down **10 times.**

3 Cap the tube. Incubate samples at **room temperature** (15-30°C) for **20 minutes.**

4 Mix the bottle of **Bind** (VBE) to fully resuspend the beads.

5 Add **350 µL** of **Bind** (VBE) to the samples and mix by vortexing the tube. Incubate samples at **room temperature** (15-30°C) for **5 minutes**.

Shake **Bind** (VBE) to disperse beads before adding to sample.

6 Place tubes on a SPRIstand and separate for **10 minutes**.

7 Fully remove supernatant from tube and discard.

This step must be performed while the tube is situated on the magnet stand.

8 Remove the tube from the magnet and wash by adding **400 µL** of **70% Ethanol** to the sample. Pipette mix 10 times, or until well mixed.

9 Place tube on the magnet and separate for **2 minutes**. Wait for the solution to clear before proceeding to next step.

10 Completely remove supernatant from the tube and discard. This step must be performed while the tube is situated on the magnet. Do not disturb the ring of separated magnetic beads.

11 Repeat step 8 through 10 one more time for a total of two **Ethanol** washes.

12 Allow magnetic beads to dry for **1 minute** at **room temperature** (15-30°C).

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

13 Remove the tube from the magnet stand and elute nucleic acid by dissolving the beads pellet in **40 µL** of **Nuclease-Free Water**. Pipette mix **10 times** and incubate at **room temperature** (15-30°C) for **5 minutes**.

14 Return the tube to the magnet stand for **2 minutes** and carefully transfer eluted nucleic acid away from the beads and into a fresh tube for storage.

NOTE Beckman Coulter recommends RNA samples be stored on ice for immediate use, or stored at -20 to -80°C for long-term storage.

www.beckman.com