Instructions For Use

# RNAdvance Viral

RNA Isolation
from Saliva, Nasopharyngeal or Oropharyngeal Swabs

PN C58529AA
May 2020

Beckman Coulter, Inc.
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BECKMAN COULTER

**RNAdvance Viral**
**RNA Isolation from Saliva, Nasopharyngeal or Oropharyngeal Swabs**
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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 C63510 — RNAdvance Viral

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

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

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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.*

**Initial Issue, C58529AA, 05/2020**

# Protocol for RNA Isolation from Saliva, Nasopharyngeal or Oropharyngeal Swabs

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

## Table of Contents



## Product Description

The RNAdvance Viral RNA purification kit utilizes Beckman Coulter, Inc.'s SPRI paramagnetic bead-based technology to isolate RNA from saliva, nasopharyngeal and oropharyngeal swabs. The protocol can be performed in both 96-well plate and single tube formats. Purification begins with lysis and protein digestion. 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 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 or saliva per well in 96-well plate and 1.5 mL tube formats.



PN C58529AA

## Kit Specifications

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

| Kit Part Number | Number of Preps |
|---|---|
| C63510 | 768 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 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

- Wear appropriate personal protection equipment while handling the samples and reagents in this protocol.
- 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 use reagents beyond the expiration date on the vial label.
- Use Good Laboratory Practices (GLP) and follow laboratory, local, or national safety guidelines when handling any virus or reagent.
- Do not mix reagents or waste from this kit with bleach. Harmful gases may be released.


| | |
|---|---|
| **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** | |
| **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*. |

| | | |
|---|---|---|
|  | **WARNING** | |
| | **Wash WBE:** Guanidine Thiocyanate 30 – 40% | |
| | Polyoxyethylated Octyl Phenol 1 – 2% | |
|  | **H302** | 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 *www.beckman.com/techdocs*. |

## Storage and Stability

**NOTE** Refer to the product labels for expiration dates.

| Reagent | Storage Condition |
|---|---|
| **Lysis LBF** | **15 - 30°C** |
| **Bind BBD** | **15 - 30°C** |
| **Wash WBE** | **15 - 30°C** |
| **Proteinase K** | **-15 to -25°C** |
| **PK Buffer** | **15 - 30°C** |

## Materials Supplied

| Reagent | 768 Preps Kit (C63510) | Symbol |
|---|---|---|
| **Lysis LBF** | REF **C42153** |   |
| **Bind BBD** | REF **C42156** |   |

| Reagent | 768 Preps Kit (C63510) | Symbol |
|---|---|---|
| **Wash WBE** | REF **C42172** |   |
| **Proteinase K** | REF **C42176** | – |
| **PK Buffer** | REF **C42150** | – |

# Materials Required but not Supplied

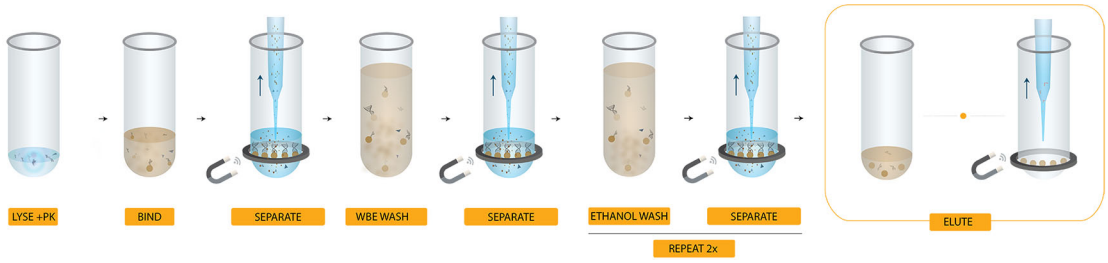
## Consumables and Hardware

| 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 |
|---|---|
| **100% Isopropanol** | – |
| **70% Ethanol**, Made With **Nuclease-Free Water**<br><br>**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 **Proteinase K** and **Lysis** (LBF) to sample and mix.
2. Lysis and **Proteinase K** digestion.
3. Addition of **Bind** (BBD).
4. Magnetic separation of beads from supernatant, wash with **Wash** (WBE).
5. Magnetic separation of beads from supernatant, wash with **Ethanol**.
6. Elution.

## Procedure

### Part A — Reagent Preparation

Prepare the following reagents in advance for both the 96-well and 1.5mL tube protocols:

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**1** Add **10mL** of **PK Buffer** to the **Proteinase K** tube/bottle. (Final concentration is **50 mg/mL**):

Mix components by inverting the tube/bottle several times. *To avoid foaming, do not vortex.* Solution will appear cloudy immediately after mixing. Let the solution sit for **5 minutes** to clear prior to using. **Store the Proteinase K solution at -20°C when not in use.**

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**2** Add **225 mL** of **100% Isopropanol** to **Wash** (WBE) bottle.

Following the **Isopropanol** addition, write the data on the label of the bottle and store the **Wash** (WBE) at **room temperature**.

**3** Prepare **Bind** (BBD) **/Isopropanol** solution:

**205 µL** of **Bind** (BBD) **/Isopropanol** solution is required per sample.

**a.** Vortex the tube containing **Bind** (BBD) *for at least **30 seconds*** to fully resuspend the beads.

**b.** Combine **5 µL Bind** (BBD) with **200 µL** of **100% Isopropanol** and mix thoroughly.

**IMPORTANT** *The* **Bind** (BBD) **/Isopropanol** *solution must be prepared fresh on the day of the experiment.* Discard any unused portion.

## Part B — RNAdvance Viral 96-Well Plate Protocol

**Viral Transport Media:** Samples 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.

**Saliva:** Samples should be stored per manufacturer's instructions. If frozen, thaw samples completely before transferring. Avoid transferring any particulate matter into the sample plate.

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** Aliquot **200 µL** of **sample** into each well of a 1.2 mL processing plate.

**2** Add **Proteinase K** and **Lysis** (LBF):

- Add **10 µL** of **Proteinase K** (**50 mg/mL**, prepared in Part A — Reagent Preparation, step 1)
- Add **150 µL** of **Lysis** (LBF)

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

**3** Lysis and protein digestion:

Seal plate with a plate seal. Incubate samples at **room temperature** for **20 minutes**.

**4** Add **205 µL** of the **Bind** BBD **/Isopropanol** solution (prepared in Part A — Reagent Preparation, step 3) to the samples and pipette mix **10 times** or until well mixed. Incubate samples at **room temperature** for **5 minutes**.

Shake or tip-mix **Bind** BBD **/Isopropanol** solution to disperse beads before adding to sample.

**5** Place 1.2 mL processing plate on SPRIPlate 96R Super Magnet Plate and separate for **5 minutes**.

**6** 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.

**7** Remove the 1.2 mL processing plate from the magnet and wash the beads by adding **400 µL** of **Wash** WBE. (**Isopropanol** must be added to **Wash** WBE before using the kit for the first time – see Part A — Reagent Preparation, step 2).

Pipette mix **10 times** to resuspend the magnetic beads.

**8** Place 1.2 mL processing plate on the magnet and separate for **5 minutes**.

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

**9** 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.

**10** Wash by adding **400 µL** of **70% Ethanol**. ***Do Not Pipette Mix.*** Let sit for approximately **2 minutes** and then remove **Ethanol** while processing plate remains situated on the magnet plate.

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

**12** Allow magnetic beads to dry for **1 minute** at **room temperature**.

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 RNA by adding **40 µL** of **Nuclease-Free Water**. Pipette mix **10 times** and incubate at **room temperature** 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 C — RNAdvance Viral 1.5 mL Tube Protocol

**Viral Transport Media**: Samples 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.

**Saliva**: Samples should be stored per manufacturer's instructions. If frozen, thaw samples completely before transferring. Avoid transferring any particulate matter into the sample plate.

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** Aliquot **200 µL** of sample into a 1.5 mL microcentrifuge tube.

**2** Add **Proteinase K** and **Lysis** (LBF):

- Add **10 µL** of **Proteinase K** (50 mg/mL, prepared in Part A — Reagent Preparation, step 1)
- Add **150 µL** of **Lysis** (LBF)

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

**3** Lysis and protein digestion:

Cap the tube. Incubate samples at **room temperature** for **20 minutes.**

**4** Add **205 µL** of **Bind** (BBD) **/Isopropanol** solution (prepared in Part A — Reagent Preparation, step 3) to the samples and mix by vortexing the tube. Incubate samples at **room temperature** for **5 minutes.**

Shake or tipmix **Bind** (BBD) **/Isopropanol** solution to disperse beads before adding to sample.

**5** Place tubes on a SPRIstand and separate for **5 minutes.**

**6** Fully remove supernatant from tube and discard.

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

**7** Remove tube from the magnet stand and wash the beads by adding **400 µL** of **Wash** (WBE).

(Isopropanol must be added to **Wash** (WBE) before using the kit for the first time – Part A — Reagent Preparation, step 2.)

Pipette mix **10 times** to resuspend the magnetic beads.

**8** Place the tube on the magnet stand and separate for **5 minutes.**

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

**9** Completely remove supernatant from the tube and discard.

This step must be performed while the tube is situated on the magnet stand. Place the pipette tip at the bottom of the tube and carefully aspirate the liquid so the layer of separated magnetic beads on the side of the tube is not disturbed.

**10** Wash by adding **400 µL** of **70% Ethanol**. ***Do Not Pipette Mix.*** Let sit for approximately **2 minutes** and then remove the **Ethanol** while the tube remains situated on the magnet stand.

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

**12** Allow magnetic beads to dry for **1 minute** at **room temperature**.

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 RNA by dissolving the beads pellet in **40 µL** of **Nuclease-Free Water**. Pipette mix **10 times** and incubate at **room temperature** 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 -2 to -80°C for long term storage.

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