

ArcPure™

Viral DNA Isolation and Sample Preparation Kit

**Optimized for the extraction and purification of viral DNA from whole
blood and plasma samples**

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For Research Use Only



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ArcPure™ Viral DNA Isolation and Sample Preparation Kit

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INTENDED USE

This protocol is for purification of viral DNA from up to 200 µl of whole blood or plasma using the ArcPure™ Viral DNA Isolation and Sample Preparation Kit and a microcentrifuge.

PROCEDURE OVERVIEW

The ArcPure™ Viral DNA Isolation and Sample Preparation Kit is designed to isolate and purify viral DNA from whole blood and plasma (sodium citrate or EDTA anticoagulants). The kit combines the selective binding properties of a silica-based membrane with flexible sample input volumes up to 200 µl. With an elution volume range between 20 and 150 µl, the kit can perform a 10X concentration of a viral DNA-containing sample. The procedure utilizes ArcPure™ Viral DNA columns and a standard microcentrifuge. The procedure includes the steps of cell lysis, nucleic acid binding to the column, washing away of unbound materials, and elution of purified DNA from the column. The procedure is designed to prevent sample-to-sample cross-contamination and provides for safe handling of potentially infectious samples. The ArcPure™ procedure is suitable for processing multiple samples and yields pure, isolated nucleic acid within 30 minutes. The ArcPure™ Viral DNA Isolation and Sample Preparation Kit can be used for isolation of viral DNA from a broad range of DNA viruses.

PRINCIPLES OF THE PROCEDURE

Whole blood or plasma samples are lysed under extreme denaturing conditions. Lysis is performed by a combination of Proteinase K and Viral DNA Lysis Buffer, thereby ensuring inactivation of RNAses and DNases. Lysates are subsequently mixed with alcohol, transferred onto purification columns and viral nucleic acids are adsorbed onto the membrane as the lysis solution is drawn through the column by centrifugation.

The alcohol concentration of the reagents is adjusted to allow optimal binding of the viral DNA to the column membrane. Fixed pH and salt concentration assures that protein and other contaminants capable of inhibiting PCR and other downstream enzymatic reactions are not retained. Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away in three wash steps. Finally, high purity viral DNA is eluted from the column using a PCR compatible solution.

Carrier Nucleic Acid enhances binding of viral nucleic acids to the *ArcPure*[™] Viral DNA column material, particularly if there is a very low target molecule population in the sample. If Carrier Nucleic Acid is not added to the Viral DNA Lysis Buffer, reduced viral DNA recovery may result. The amount of lyophilized Carrier Nucleic Acid (provided) is sufficient for the volume of Viral DNA Lysis Buffer provided. The concentration of Carrier Nucleic Acid has been optimized as a generic purification system compatible with many different amplification systems and is therefore suitable for a wide variety of DNA viruses. Amplification systems vary in efficiency depending on the total amount of nucleic acid introduced. *ArcPure*[™] eluates contain both viral nucleic acids and carrier nucleic acid, with the latter greatly exceeding the former. Calculations of how much eluate to add to downstream amplifications should be based on the amount of Carrier Nucleic Acid added to the Viral DNA Lysis Buffer. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of Carrier Nucleic Acid used.

Use of the *ArcPure*[™] Viral DNA sample preparation, in combination with commercially available amplification methods, may require the use of internal control(s). An internal control may be added together with the Carrier Nucleic Acid to the Viral DNA Lysis Buffer. Internal control molecules should be longer than 200 nucleotides, due to inefficient recovery of smaller molecules. Refer to the control manufacturer's instructions for the optimal concentration and maximum amplification efficiency.

ArcPure[™] Viral DNA columns are suitable for use in standard microcentrifuge tubes. The required 2 ml collection tubes (provided) support the *ArcPure*[™] Viral DNA column during the loading and wash steps.

The *ArcPure*[™] Viral DNA columns provide for elution volumes as low as 20 µl. These minimal elution volumes lead to highly concentrated nucleic acid eluates. Downstream applications requiring greater starting volume (e.g. specific PCR/RT-PCR) can be accommodated by increasing the elution volume accordingly. The increase in elution volume however, will decrease the nucleic acid concentration in the eluate. The eluate volume recovered may reach a volume up to 5 µl less than elution buffer volume applied (i.e. use of 20 µl of elution buffer may result in <15 µl final eluate). Recovered eluate volume is related to the nature of the sample.

Because the amount of nucleic acid isolated from biological samples is typically < 1 µg, yield is difficult to determine via spectrophotometry. Quantitative amplification methods are recommended for determination of yields. When quantifying nucleic acids isolated using the *ArcPure*[™] sample preparation protocol there will be considerably more carrier nucleic acid present compared with viral DNA. The size distribution of viral nucleic acid purified with this procedure can be verified utilizing agarose gel electrophoresis and hybridization to a virus-specific labeled probe¹.

KIT MATERIALS PROVIDED

The *ArcPure*[™] Viral DNA Isolation and Sample Purification Kit contains sufficient reagents to process 100 whole blood or plasma specimens. Each kit contains the following materials:

***ArcPure*[™] Viral DNA Extraction Columns (100 each)**

Collection Tubes (2 bags of 50 tubes)

Viral DNA Lysis Buffer (25 ml)

Viral DNA Wash Buffer (17.2 ml concentrate)

Proteinase K (1 vial)

Carrier Nucleic Acid (1 vial)

KIT STORAGE AND HANDLING

- *ArcPure*[™] Viral DNA columns can be stored at room temperature (15-27°C) for extended periods of time.
- All *ArcPure*[™] Sample Preparation Buffers can be stored at room temperature (15-27°C) for extended periods of time.
- It is recommended to store Proteinase K and Carrier Nucleic Acid at 2-8°C upon arrival.
- It is important to track kit contents when storing kit components separately.

KIT QUALITY CONTROL

In accordance with Arcxis' Quality Management System, each lot of *ArcPure*[™] Viral DNA Isolation and Sample Preparation Kits is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in a diagnostic procedure.
- All biological specimens should be treated as if they are capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all human specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention².
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- All hazardous materials should be disposed of according to your laboratory's safety guidelines³.

SAFETY

When working with ArcPure™ reagents, always wear suitable personal protective equipment (PPE) including lab coat, disposable gloves, and protective goggles. For more complete safety information, consult the appropriate Arcxis Biotechnologies material safety data sheets (MSDS).

Viral DNA Lysis Buffer and Viral DNA Wash Buffer each contain guanidine based chaotropic salts. The following risk and safety phrases apply to both these buffers:

- R22: Harmful if swallowed
- R36/38: Irritating to eyes and skin
- S13: Keep away from food, drink and animal feeding stuffs
- S24: Avoid contact with skin
- S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
- S36/37/39: Wear suitable protective clothing, gloves and eye/face protection
- S46: If swallowed, seek medical advice immediately and show the container or label

CAUTION: DO NOT add bleach or acidic solutions directly to liquids containing Viral DNA Lysis Buffer or Viral DNA Wash Buffer. Both buffers contain guanidine based chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

24-hour emergency medical information in multiple languages can be obtained 24 hours a day from the Poison Information Center Sacramento, CA, USA Tel: 1-800-222-1222 or online at: www.calpoison.org.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Isopropanol (96-100%)
- Ethanol (96–100%)
- Nuclease-free water
- Elution Buffer (Nuclease-free water or Tris-EDTA, pH 8)
- 0.9% NaCl solution (optional)
- 1.5 ml microcentrifuge tubes (e.g., Eppendorf® Safe-Lock, cat. no. 022463204) for incubations and elution
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- 60°C Heating block (optional)
- Microcentrifuge capable of speeds up to 20,000 x g (14,000 rpm) with rotor for 1.5 ml and 2 ml tubes
- Vortexer

SPECIMEN COLLECTION AND TRANSPORT

Collect peripheral blood specimens according to standard techniques at each institution. Collect blood specimens in tubes containing EDTA or sodium citrate as anticoagulant. Store at 2-8°C and use within 8 hours of collection. For long-term storage, freezing at –20°C or –80°C in aliquots is recommended. Frozen blood or plasma samples must not be thawed more than once as it leads to denaturation and precipitation of proteins, resulting in reduced viral titers/nucleic acid yields. Cryoprecipitates formed in freeze–thaw cycles will also clog the *ArcPure*™ Viral DNA membrane. If cryoprecipitates are visible, centrifuge at 6800 x g for 3 minutes to form a pellet and remove the clear supernatant for processing.

LIMITATIONS

Each *ArcPure*™ Viral DNA column can bind nucleic acids that are longer than 200 bases, but yield depends on sample volume and virus titer. The spin procedure is optimized for use with a starting volume of up to 200 µl of whole blood or plasma.

The *ArcPure*™ Viral DNA Isolation and Sample Preparation Kit is intended for general laboratory use. It is not intended to provide information for the diagnosis, prevention, or treatment of disease. Good laboratory practices should be exercised in the handling of the products.

PROCEDURE

IMPORTANT POINTS BEFORE STARTING

- All centrifugation steps are to be carried out at room temperature (15–25°C).
- Equilibrate samples and buffers to room temperature before beginning the procedure.
- Yield efficiency increases when the Elution Buffer is preheated to 60°C prior to adding it to the ArcPure™ Viral DNA column and by incubation for 5 minutes before centrifugation.

GENERAL HANDLING

Proper microbiological aseptic technique should always be used when working with RNA or DNA. Dust particles may carry bacteria and molds and are the most common sources of nuclease contamination. Always wear latex or vinyl gloves while handling reagents and samples to prevent nuclease contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed.

PREPARATION OF WORKING SOLUTIONS

Preparation of Carrier Nucleic Acid/ Viral DNA Lysis Buffer Mixture

Add 1.5 ml of nuclease-free water or Tris-EDTA, pH 8 buffer to the tube containing 2 mg lyophilized Carrier Nucleic Acid to obtain a solution of 1.3 µg/µl. Dissolve the Carrier Nucleic Acid thoroughly, aliquot and store at –20°C. Do not freeze–thaw more than 3 times.

On the day of use, mix the Carrier Nucleic Acid solution with Viral DNA Lysis Buffer to create a working solution. Calculate the volume of Carrier Nucleic Acid/ Viral DNA Lysis Buffer mixture needed per experiment as follows where (N) is the number of samples to be processed simultaneously:

$$N \times 208 \mu\text{l} = \text{volume of Viral DNA Lysis Buffer required } (\mu\text{l})$$

$$N \times 12 \mu\text{l} = \text{volume of Carrier Nucleic Acid solution required } (\mu\text{l})$$

Mix the calculated amounts of Carrier Nucleic Acid solution and Viral DNA Lysis Buffer together. Mix by pulse-vortexing for 10 seconds.

Note: The sample-preparation procedure is optimized for 16 µg of Carrier Nucleic Acid per sample. If less Carrier Nucleic Acid is recommended for your amplification system, transfer only the required amount of dissolved Carrier Nucleic Acid to the tubes containing the Viral DNA Lysis Buffer. Use of less than 16 µg Carrier Nucleic Acid per sample must be validated by the user.

Preparation of Working Viral DNA Wash Buffer

Add 40 ml of ethanol (96–100%) to the 17.2 ml of solution in the Viral DNA Wash Buffer bottle. Annotate the bottle label to indicate that ethanol has been added. Store the Working Viral DNA Wash Buffer at room temperature (15–25°C). The Working Viral DNA Wash Buffer is stable for up to 1 year when stored at room temperature.

Note: Always mix Working Viral DNA Wash Buffer thoroughly prior to use.

Preparation of 80% Ethanol Solution

Mix 30 ml of ethanol (96–100%) with 7.5 ml of the nuclease-free water. Label the bottle as 80% Ethanol Solution. Store the 80% Ethanol Solution at room temperature (15–25°C). The 80% Ethanol Solution is stable for up to 1 year when stored at room temperature.

Note: Always mix 80% Ethanol Working Buffer thoroughly prior to use.

ArcPure™ Viral DNA Column Handling

The following precautions are necessary when handling ArcPure™ Viral DNA columns in order to avoid sample cross-contamination:

- Carefully apply the sample or solution to the ArcPure™ Viral DNA column by pipetting directly into the center without wetting the rim of the column. Avoid touching the ArcPure™ Viral DNA membrane with the pipette tip.
- Change pipet tips between all liquid transfers. Use of aerosol-barrier pipet tips is recommended.
- After vortexing, briefly centrifuge tubes to remove droplets from the inside lid.
- Wear gloves at all times. If gloves contact the sample, change gloves immediately.

VIRAL DNA ISOLATION PROCEDURE

1. Pipet 20 µl Proteinase K solution into a 1.5 ml centrifuge tube (not provided).
2. Add up to 200 µl of plasma or whole blood into the tube. **IMPORTANT NOTE:** Ensure that plasma or blood samples are equilibrated to room temperature prior to purification. If sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease/sample mixture up to a total of 220 µl.
3. Close the cap of the tube and mix thoroughly by pulse-vortexing for 10 seconds.
4. Incubate the Proteinase K/sample mixture for 10 minutes at room temperature (15–25°C).
5. Add 220 µl Carrier Nucleic Acid/ Viral DNA Lysis Buffer mixture (prepared ahead of time). Close the cap of the tube and mix by pulse-vortexing for 10 seconds. In order to ensure efficient lysis, it is essential that the sample and Viral DNA Lysis Buffer mixture are mixed thoroughly to yield a homogeneous solution.
6. Incubate the sample/ Viral DNA Lysis Buffer mixture for 10 minutes at room temperature (15–25°C).
7. Add 310 µl of Isopropanol (96–100%) to the sample lysate. Close the cap of the tube and mix thoroughly by pulse-vortexing for 10 seconds. Incubate the lysate/Isopropanol mixture for 5 minutes at room temperature (15–25°C).

Note: If ambient temperature exceeds 25°C, the Isopropanol should be cooled on ice before adding to the lysate.

8. Place the *ArcPure*[™] Viral DNA column into one of the microcentrifuge collection tubes (supplied). Carefully apply all of the lysate/Isopropanol mixture onto the *ArcPure*[™] Viral DNA column without wetting the rim. Centrifuge at 6000 x g (8000 rpm) for 1 minute. The entire sample can be fully loaded in one column load.
9. Remove the *ArcPure*[™] Viral DNA column and collection tube from the microcentrifuge. Discard the filtrate and carefully place the column back into the collection tube.
Note: The filtrate contains hazardous waste and should be disposed of appropriately.
10. Add 500 µl of Working Viral DNA Wash Buffer without wetting the rim and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discard the filtrate and carefully place the column back into the collection tube.
11. Add 500 µl of 80% Ethanol without wetting the rim and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discard the filtrate and carefully place the column back into the collection tube.
12. Add an additional 500 µl of 80% Ethanol without wetting the rim and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discard the filtrate and carefully place the column back into the collection tube.
13. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes to dry the membrane completely.
14. Place the dry *ArcPure*[™] Viral DNA column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube. Apply 60 µl (20–150 µl) of pre-heated Elution Buffer to the membrane. If elution is performed in small volumes (<50 µl), it is critical that the Elution Buffer be dispensed onto the center of the membrane for complete elution of bound DNA. Incubate at room temperature for 5 min. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.
Note: The final elution volume is flexible and can be adapted according to the requirements of the downstream application. The recovered eluate volume will be approximately 5 µl less than the elution buffer volume applied onto the column. Incubating the *ArcPure*[™] Viral DNA column loaded with 60°C preheated Elution Buffer for 5 minutes at room temperature before centrifugation generally increases DNA yield.
15. Discard column and store eluted purified DNA as appropriate. The purified nucleic acids are free of proteins, nucleases, and other impurities and can be stored at –20°C for prolonged periods of time without sample degradation. Eluted DNA can be stored for up to 24 hours at 2–8°C. Storage at –20°C is recommended for periods longer than 24 hours.

BIBLIOGRAPHY

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2. US Centers for Disease Control. *Morbidity and Mortality Weekly Review*. 1987;36(sup. 2S):2S-18S.
3. NIOSH Publication No. 2005-151: *NIOSH Pocket Guide to Chemical Hazards*, September 2005.

TECHNICAL ASSISTANCE

The Arcxis Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Arcxis products. If you have any questions or experience any difficulties regarding the ArcPure™ Viral DNA Isolation and Sample Preparation Kit or Arcxis products in general, please do not hesitate to contact us.

Every product at Arcxis Biotechnologies begins with the customer. Arcxis prides itself on working with our customers collaboratively to improve and develop new products. If you have comments or suggestions, please contact the company by email (techsupport@arcxis.com) or by phone (+1-925-621-7951).

PRODUCT WARRANTY

Arcxis guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. We reserve the right to change, alter or modify any product to enhance its performance and design. If an Arcxis product does not meet your expectations, please contact the company by email (customersupport@arcxis.com) or by phone (+1-925-621-7950).

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