Viral Prep Adem-Kit

(Cat #06230)

Instruction for manual protocol

The kit is used for optimal viral RNA/DNA extraction from body fluids such as blood, serum or plasma, as well as from cells culture supernatants or stools.

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Introduction

1. Description

The Viral Prep Adem-Kit is specially designed for optimal viral RNA/DNA extraction from body fluids such as **blood**, **serum** or **plasma**, as well as from **cells culture supernatants** or **stools**. The Viral Prep Adem-Kit technology is based on the adsorption of RNA/DNA to paramagnetic particles under appropriate conditions. The Viral Prep Adem-Kit was developed to improve RNA/DNA extraction from all virus species (including enveloped or naked virus) for molecular biology applications.

2. Product Component and storage conditions

Kit Content: Each Viral Prep Adem-Kit contains sufficient reagents to perform 96 samples using the following standard protocol.

Item	Viral Prep Adem-Kit
Cat No.	06230
Package size	96 samples
LB Buffer	40 mL
Carrier	0,2 mL
Prep-Adembeads	3.6mL
Viral Washing Buffer I (WB)	3X28mL
Washing Buffer II	2X17 mL
Elution Buffer	10 mL

Viral Prep Adem-Kit (#06230)	
Reagents	**Storage condition**
LB Buffer	+4-25°C
Carrier	+4-25°C
Prep-Adembeads	+4-25°C
Viral Washing Buffer I (WB)	+4-25°C
Washing Buffer II	+4-25°C
Elution Buffer	+4-25°C

Storage conditions: The kits are shipped at room temperature.

NOTE 1! Properly stored Kits are guaranteed until the expiry date. Note that shipping is realized at room temperature and will not affect stability. All components of the kit have been prepared under nucleases free conditions and have been thoroughly tested to ensure optimal performance.

NOTE 2! Storage conditions

All reagents in the kit can be stored in the range of temperatures from +4°C to +25°C. The carrier also can support this storage conditions. This will not affect the stability of this reagent.

If you store the whole kit at +4°C, before using, it is recommended to take out the reagents in advance and check if there are any precipitates. If the Buffers present precipitates place them at room temperature and eventually put them at +37°C.

IMPORTANT! Do not freeze the magnetic particles.

3. Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. To avoid contamination of your sample, wear face mask.

Reagents:

- Isopropanol
- 96-100% ethanol
- 70% ethanol

Materials:

- 50°C heat block, thermomixer or water bath
- Microtubes
- **Adem-Mag MODULO** (Cat.# 20105, # 20108)

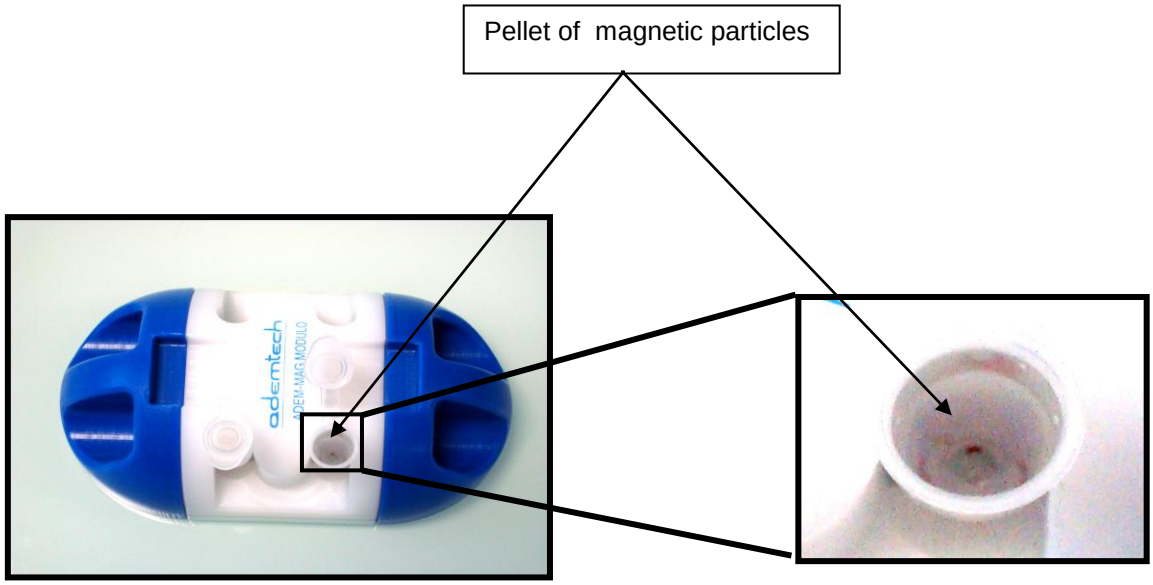
Viral Prep Adem-Kit Protocol

1. Prep-Adembeads Guidelines

- Before using Prep-Adembeads, thoroughly flick / vortex the bottle to completely resuspend the magnetic particles.

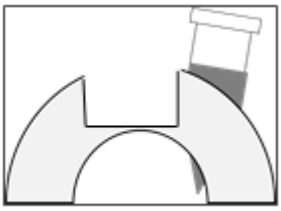
- During separation steps, let the microtubes containing magnetic particles on the magnet 1-5 minutes depending of the media. The magnetic particles pellet is oriented toward the magnet at the back of the microtubes.

- When removing the liquid phase, pipette off carefully, do not aspirate magnetic particles or disturb the magnetic pellet



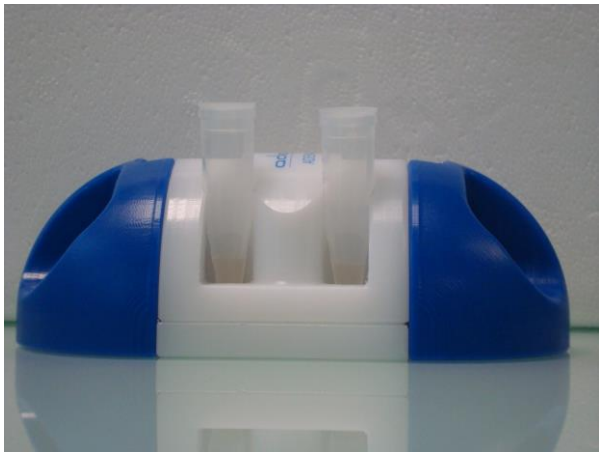
2. Magnetic Stand Guidelines

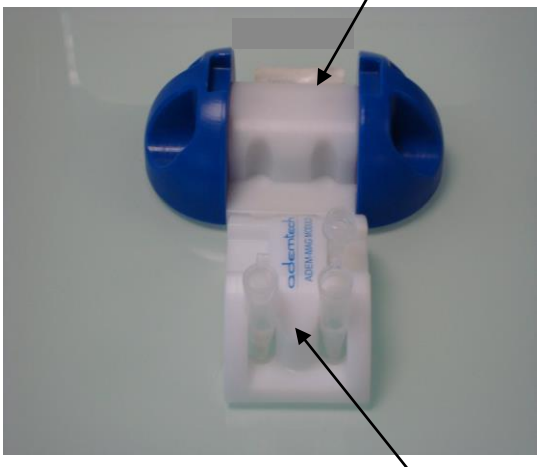
- Place magnetic base away from metal objects/magnetic media.
- Insert the microtubes into the sample holder in the correct position.



Correct position

- Insert sample holder into magnetic base. To help optimize magnetic pellet formation ensure that the magnetic stand is correctly assembled before performing washing and elution step.
- The sample holder can be quickly removed from the magnetic base to resuspend the beads.





3. Viral Prep Adem-Kit Protocol

The standard protocol is appropriate for all kind of virus species present in **blood**, **serum** or **plasma** samples, as well as from **cells culture supernatants** or **stools**. The protocol is also suitable for viral RNA/DNA extraction from up to 250µl of sample.

3.1. Reagents preparation

 **WARNING ! CHEMICAL HAZARD. LB Buffer and Viral Washing Buffer I** in contact with acids or bleach liberate toxic gazes. Harmful if inhaled, absorbed through the skin, and swallowed. Cause eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapour. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow and handling instructions. Wear appropriate protect eyewear, clothing and gloves.

- Prepare a 70% ethanol solution.

- Prepare the Viral Washing Buffers I (WB) & Washing Buffer II before first use:
  1. Add **28mL of 96-100% ethanol to each bottle containing 28mL of Viral Washing Buffer I (WB). Homogenize the solution.**
  2. **Add 39mL of 96-100% ethanol to each bottle containing 17mL of Washing Buffer II. Homogenize the solution.**

NOTE! Viral Washing Buffer I (WB) and Washing Buffer II are delivered concentrated. Before the first use, you have to **add ethanol** in the bottle in the indicated proportion.

3.2. Perform viral lysis

1. Place the sample (**up to 250µl**) in a 1,5ml or 2ml microtube.

NOTE to perform extractions from cell culture!

You have to centrifuge the sample 3 minutes at 2.000rpm in order to clarify the supernatant.

NOTE to perform extractions from stools!

One ml of stool samples (up to 200mg), fresh or freezed, is diluted with 5ml of NaCl 0,89% (physiological water). The sample is centrifuged 20 min at 4.000g and 250µl of supernatant are used for nucleic acids extraction.

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2. Add 400µl of LB Buffer in the microtube containing the sample.

3. Add 2µl of carrier.

NOTE! The carrier will be co-extracted with viral RNA/DNA. So, if the carrier interferes with your downstream applications, do not use it.

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4. Close the tube and mix well with a vortex. Place the tube in a thermomixer and incubate at room temperature for 10 minutes at 1000rpm.

3.3. Bind viral RNA/DNA

1. To the lysate, add 365µL of isopropanol and 35µL of Prep-Adembeads. Mix well with a vortex.

NOTE! It is possible to prepare a premixed solution of Isopropanol / Prep-Adembeads.

Isopropanol	365µL	⎤	X number of extractions
Prep-Adembeads	35µL	⎦	

Add 400µL of premixed solution.

2. Place the tube in a thermomixer and incubate at room temperature for 5 minutes at 1000rpm.

NOTE! During capture of RNA/DNA, it is important to shake in order to improve interactions between nucleic acids and particles.

NOTE! During the incubation, two phases may form in the mixture but without effect on the nucleic acids capture.

3.4. Wash bound RNA/DNA

After binding RNA/DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors. In this protocol, there are **four consecutives washes**.

NOTE! In a general manner, during washing steps, magnetic beads aggregations may occur depending of the initial sample. For example, more aggregations can be observed with blood than with cells culture supernatant. Resuspend magnetic beads with a vortex without trying to break aggregates.

Washing 1

a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in 800µL of Viral Washing Buffer I (with ethanol).

Washing 2

c. Magnetize the particle suspension at least 1 minute, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

d. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in 800µL of Viral Washing Buffer I (with ethanol).

Washing 3

a. Magnetize the particle suspension at least 1 minute, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in 800µL of Washing Buffer II (with ethanol).

NOTE! From Washing step 3, it is possible that magnetic beads adhere to the microtube. In this case, do not try to unstuck beads. They were release at the elution step.

Washing 4

a. Magnetize the particle suspension at least 1 minute, and discard carefully the supernatant without disturbing the pellet of magnetic particles.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in 800µL of 70% ethanol.

3.5. Drying

1. Magnetize the particle suspension at least 1 minute.

2. Eliminate carefully the supernatant without disturbing the pellet of magnetic particles.

3. Let the pellet of magnetic particles dry for 5 minutes.

NOTE! Magnetization and Drying times are given as an indication.

The drying time may be reduced to facilitate the recovery of the pellet.

3.6. Elute RNA/DNA

1. Remove the microtube from the magnet and resuspend thoroughly the pellet of magnetic particles in 60-100µL of Elution Buffer.

2. Place the tube in a thermomixer and incubate at 50°C for 5 minutes at 1000rpm.

3. Place the microtube on the magnet for at least 3 minutes.

4. Collect the supernatant containing pure RNA/DNA and transfer it to a new clean microtube.

NOTE! If nucleic acids are not analyzed immediately store them at -20°C.

Troubleshooting

Observations	Possible cause	SUGGESTION
Magnetic particles settled in the bottle.	During shipping, magnetic particles settled.	Thoroughly flick / vortex the bottle. Prep-Adembeads are stored between+2-8°C, before using incubate them at room temperature.
Supernatants contain magnetic particles.	The magnetic stand used is not adapted to the magnetic particles. Incorrect position for microtubes in the sample holder.	Keep the tube containing magnetic particles in the magnet for at least 5 minutes.
Elution contains magnetic particles	Aggressive pipetting can disturb magnetic pellet.	Keep the tube containing magnetic particles in the magnet for at least 5 minutes then pipette out carefully the supernatant.
Nor or low yield of RNA/DNA	Biological sample contains no or low amount of RNA/DNA.	Review protocol steps and reagents additions.
		Repeat RNA/DNA extraction with a new concentrated sample.
		Review protocol steps and reagents additions.

Warranty

This product is only for use in research. The purchaser is responsible to validate the performance of this product for any particular use, and to use the product in compliance with any applicable regulations.
The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life. Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price. Claims for merchandise damaged in transit must be submitted to the carrier.

Ordering Information

- **Ademtech Kits**

CAT NO.	PRODUCT	PACKAGE SIZE
06230	Viral Prep Adem-Kit	96 preps
06231	Viral Prep Adem-Kit AutoMag Solution (prefilled reagents plates)	48 (12X4)

- **Instrument and consumables**

CAT NO.	PRODUCT	PACKAGE SIZE
20105	Adem-Mag MODULO Classic	Each
20106	Adem-Mag 96	Each
20108	Adem-Mag MODULO Brick	Each