

For research use only

INTRODUCTION

The kit is based on the use of the **Biomagnetic separation technology**. The separation method is gentle and does not require the use of columns or centrifugation step.

Biomagnetic separation technology is a simple technique based on the separation of **superparamagnetic beads** using a magnetic field. When added to a complex medium, the **Viro-Adembeads** will bind to the target. This interaction is based on the affinity of the polyelectrolyte layer adsorbed on the surface of the beads with virus. The resulting beads-target complex can be removed from the suspension using a magnet. The benefits of magnetic handling are easy washing, separation and concentration of the target without any need for centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

VIRO-ADEMBEADS PRINCIPLE

Viro-Adembeads are designed for **simple and rapid capture and culture of viruses**. Following incubation in medium containing virus, Viro-Adembeads can be recovered by magnetic separation and directly used for cell infection. **Virus bound** onto Viro-Adembeads **is viable and retains infectivity**. The beads-virus complex suspension is mixed with cell suspension and culture is continued until analysis. **Viro-Adembeads** allows to **improve virus infection efficiency by concentrating viruses and**

promoting cell-virus contact. Viro-Adembeads also **facilitate** commonly used **viral infection protocols** that are time and labor intensive.

Virus capture is based on the interaction of **Viro-Adembeads** with the virus. The mechanism of virus capture is based on electrosteric interactions.

PRODUCT DESCRIPTION

The Kit contains all the components required for viral capture. The kit is provided to perform 25 experiments with Viro-Adembeads. Buffer is produced under aseptic conditions.

	Amount	Component	Storage
R1	1250 µl	Viro-Adembeads	+ 2-8°C
R2	5 ml	Binding Buffer	+ 2-8°C

Table 1: Components provided with the beads

GENERAL GUIDELINES

Viro-Adembeads may be applicable for the capture and culture of various viruses; it is up to you to determine the suitability for your particular use. Capture efficiency and infection depends on the starting material.

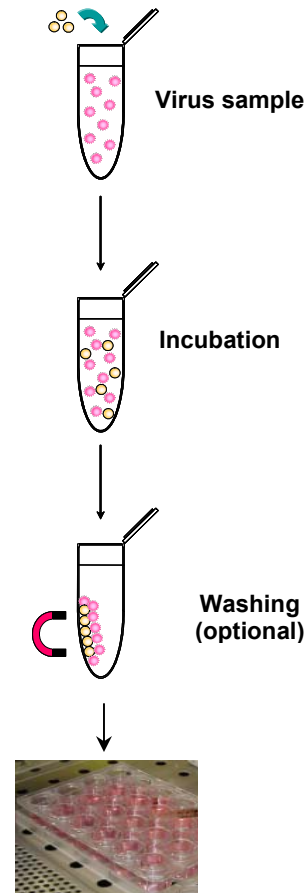
Capture and culture have been validated in the following models of enveloped viruses (Table 2).

Virus	Target cell	Infection evaluation
HIV-1	PBMC	qRT-PCR from culture supernatants
Influenza A (H1N1)	MDCK	Hemagglutination from culture supernatants
CMV	MRC-5	Cytopathic effect

Table 2: Capture and Culture of various viruses.

PROTOCOL SUMMARY

Viro-Adembeads



INSTRUCTIONS FOR USE

The protocol has been optimized for use with HIV-1 (starting from infected plasma or HIV-1 spiked serum free medium), H1N1 and CMV.

We recommended starting with the experimental procedure described below ; then you can optimize parameters such as starting material/beads and virus/beads ratios.

A) Preparation of Viro-Adembeads

1. Pipet 50 µl **Viro-Adembeads** into a 1.5 ml centrifuge tube.
2. Place the tube on the magnet for 2 minutes.
3. Remove the supernatant and resuspend particles in 50 µl Binding Buffer.
4. Repeat once step 2 and 3.
5. Place the tube on the magnet and resuspend beads in 25 µl Binding buffer.

B) Sample preparation

1. Prepare sample or medium containing virus according to your own application.
2. Capture protocol is performed from 500 µl starting material in 1.5 ml centrifuge tubes.

C) Virus capture protocol

1. Pipet 25 µl particles obtained in step A.5 into a 1.5 ml centrifuge tube.
2. Add 500 µl starting material and mix by pipetting (**DO NOT VORTEX**).
3. Incubate by mixing at 900 rpm and 20°C for 20 minutes.
4. Place the tube on the magnet for 1 minute and remove the supernatant.

D) Beads-virus complex washing

1. Remove the tube from the magnet and resuspend particles in 500 µl serum free medium.
2. Place the tube on the magnet for 1 minute and remove the supernatant.

E) Beads resuspension

Resuspend particles in 50 µl serum free medium

D) Recommendations for infection

Notes:

- The beads-virus complex obtained in step E can be directly used to infect cells.
- For cell growing in suspension, best results are achieved when cell pellet is resuspended with the beads-virus complex (in small

volume) and transferred into cell culture well with appropriate medium.

- For adherent cells, the beads virus complex could be directly added to the culture well.
- Pre-Incubation step of cells with virus to promote virus-cell interaction in reduced volume is not needed nor recommended with Viro-Adembeads.
- Viro-Adembeads are not toxic and cell culture can be performed in the presence of beads (beads sedimentation in the well is normal).
- If beads are to be removed from cell culture, it can be performed by magnetic separation.
- The washing step is critical when starting with plasma sample (or serum containing medium); it could be removed when starting material are PBS or serum free medium.

TROUBLESHOOTING

Beads aggregation:

Comment	Suggested
Increased standing in magnetic separator could induced aggregation.	Reduce the time of standing in magnetic separator and avoid beads standing without liquid on the magnet.

Beads sedimentation post capture:

Comment	Suggested
Increased times could lead to beads sedimentation; this have no effect on cell infection.	Be sure to fully resuspend beads before using for infection.

Clotting with beads following capture:

Comment	Suggested
Starting with plasma sample could induce clotting following step E.	Do not remove washing step (D) and if necessary proceed two washing steps. Dilute starting material.

No or low virus capture:

Comment	Suggested
Virus capture efficiency depends on the protein concentration in the starting medium.	Use protein free medium or reduce protein concentration in the starting material by dilution.
Reducing beads concentration will reduce virus capture efficiency.	Be sure to use not less than 50µl Viro-Adembeads : at step 1 be careful to fully resuspend the beads if sedimentation has occurred and pipet the correct amount of beads

ADDITIONAL MATERIAL REQUIRED

- Magnetic devices
 - Adem-Mag SV, 1.5 ml (# 20101)
 - Adem-Mag MSV, 12X1.5-2ml (#20104)
- Microtubes
- Rotation device

STORAGE / STABILITY

Properly stored Kits are guaranteed until the expiration date. Note that the shipping is realized at room temperature which will not affect the stability of the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination. If cytotoxic preservatives are added they must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For research use only. Not for use in human diagnostic or therapeutic procedures.

WARRANTY

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

Product	Description	Code
Viro-Adembeads for capture and virus culture	25 prep	07010

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