Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Mag-Adenovirus Purification Kit

01/20

(Catalog # K1459-20, -100; 20 preps, 100 preps; Magnetic beads based kits; Store at 4°C)

I. Introduction:

BioVision's Mag-Adenovirus Purification Kit is used to capture, concentrate and store adenoviruses using magnetic beads. Viruses are important gene delivery tools, which have been used in gene therapy and RNAi delivery. Traditionally viruses are purified by ultracentrifugation using CsCl which is time consuming and limited to the volume of cell lysate to be processed. BioVision's Purification kit uses magnetic nanoparticles to capture viruses by electrostatic and hydrophobic interactions with 80-99% efficiency and the purification process does not need ultracentrifugation. The viruses captured on Adenovirus (AD)-magnetic beads can be used directly for multiple assays. Alternatively, viruses can be eluted with the elution buffer and used for downstream applications. The AD-magnetic beads can be combined with any adenoviruses such as Ad Type 5 (Ad5), Oncolytic Ad (Ad520).

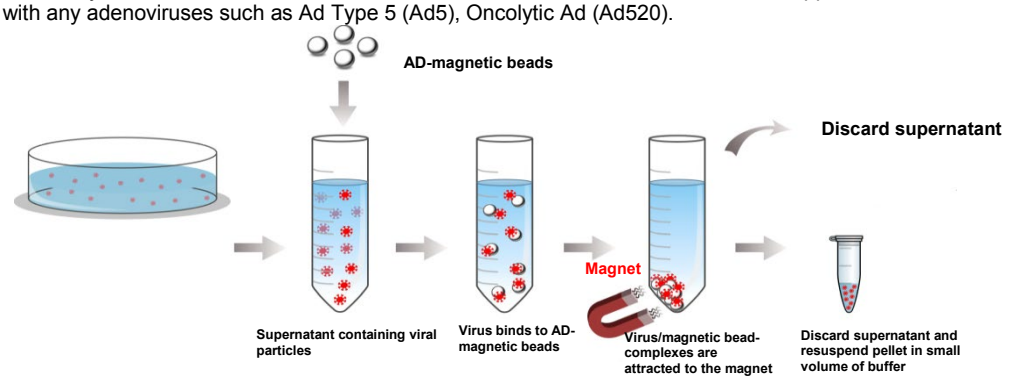

Fig.1. Protocol to capture and concentrate virus using Adenovirus (AD)-magnetic beads.

II. Applications:

PCR, Western blot, ELISA, transfection directly with viruses bound to AD-magnetic beads or eluted from the AD-magnetic beads (free of beads).

III. Key Features:

- Purify viruses in **30-45 min**
- Simple, rapid and **ready-to-use**
- **High yield** and fast concentration (2 to 1000X)
- **Does not need ultracentrifugation**
- Reduced handling steps of viruses (minimized bio-hazard)
- Suitable for **large volumes**
- **Ideal for cell culture transduction/infection**
- Improved **virus preservation** during storage at -80°C

IV. Sample Types:

- Transfected cell lines, serum

V. Kit Contents:

Components	K1459-20 (20 Preps)	K1459-100 (100 Preps)	Part Number
AD-Magnetic Beads	0.2 ml	1 ml	K1459-XX-1
Conservation Buffer (5X)	0.2 ml	1 ml	K1459-XX-2
Elution Buffer (1X)	5 ml	5 ml	K1459-XX-3

VI. User Supplied Reagents and Equipment:

- Pipettes & Pipette tips
- 1.5 ml microcentrifuge tubes
- 15 ml & 50 ml centrifuge tubes
- Magnetic separation rack
- PBS

VII. Shipping and Storage Conditions:

All kits are shipped at room temperature (RT). All kits are stored at 4°C. All reagents are stable for up to 12 months when stored properly at 4°C.

VIII. Reagent Preparation and Storage Conditions:

1. Dilute 1 volume of the Conservation Buffer (5x) in 4 volumes of PBS (1X final) freshly when required.
2. Do not freeze the AD-magnetic beads

IX. Virus Purification Protocol:

1. Add 20 µl of **AD-magnetic beads** to ≤ 2 ml virus preparation. 20 µL of magnetic beads are sufficient to bind 1 x 10^6 infectious viruses with almost 80-99% efficiency. If the virus preparation is > 2 mL add 10 µl/ml of magnetic beads.
   Note: It may be necessary to adjust the volume of magnetic beads depending on the composition of the virus solution or medium. The suggested range is 10 µl - 40 µl and 5 µl - 20 µl/ml of magnetic beads for ≤ 2 ml or > 2 mL viral preparation respectively. For high titer viral solution (≥ 10^7 infectious viruses/ml), we recommend using 1.5 or 2 times of the suggested volume.

2. Incubate for 20 - 30 min at RT to capture viruses.
3. Place the tube for 15 - 30 min on the **magnetic separation rack** to concentrate the virus/magnetic beads complexes. The incubation time will depend on the tube volume. For tube volumes of 1 ml, 10 ml, 50 ml, incubate the complexes on the magnetic rack for 15 min, 20 min and 30 min respectively. Then discard the supernatant. **Note:** Brown pellet will be visible on the side of the tube near the magnets.
4. ***Optional Washing Step:*** Keep the tube on the magnetic separation rack and slowly add an equal volume of PBS as the initial medium and incubate for 5 min. Discard the supernatant.
5. The **virus/magnetic beads complexes pellet** can be processed in 4 different ways as described below:
   a) Add small volume of **PBS** (with Ca^{2+} and Mg^{2+}) or complete cell culture medium to virus/magnetic beads complexes and use immediately for downstream applications. Determine the appropriate volume of PBS/medium to add as needed.
   b) Add small volume of **Conservation Buffer** for long term storage of virus/magnetic beads complexes.
   c) Elute viruses from magnetic beads, concentrate in small volumes of **Elution Buffer** and use immediately.
   d) Elute viruses from magnetic beads, concentrate into smaller volume of **Elution Buffer. Additionally add Conservation Buffer** for long term storage.

6. **Elution:** ***Elution step is optional.***
   a) Add the **Elution Buffer** to the virus/magnetic beads complexes pellet. To determine the appropriate volume of Elution Buffer for required concentration, please see the table below. For eg., if the initial virus solution is 1 ml and you want to concentrate 10X fold, then add 100 µl of Elution Buffer.

Table 1. Volume of Elution Buffer for concentration and immediate use

Starting Viral Solution	Required Concentration			Exchange Medium
	10X	50X	100X	
1 ml	100 µl	20 µl	10 µl	1 ml
5 ml	500 µl	100 µl	50 µl	5 ml
10 ml	1 ml	200 µl	100 µl	10 ml
50 ml	5 ml	1 ml	500 µl	50 ml

   b) After adding **Elution Buffer**, incubate for 5 to 10 min at RT.
   c) Place the tube on the **magnetic separation rack** and incubate 10 to 30 min at RT. Adjust incubation time on the magnetic separation rack according to the volume. For tube volumes of 1 ml, 10 ml, 50 ml incubate the complexes on the magnetic rack for 15 min, 20 min and 30 min respectively.
   d) **Save the supernatant containing viruses** and discard the pellet of magnetic beads.
   e) The concentrated viruses solution can be used for downstream assay or proceed to storage in step 7. The Elution Buffer does not impair viral infectious properties.

7. **Storage:**
   The Conservation Buffer allows storage of virus for several months at -80°C and preservation of the virus titer during freeze/thaw cycles. Dilute 1 volume of the Conservation Buffer (5x) in 4 volumes of PBS (1X final) freshly when required. For eg. to prepare 100 µl conservation buffer, add 20 µl of conservation buffer to 80 µL of PBS.
   Add freshly prepared **Conservation buffer** to the virus/magnetic beads complexes pellet. To concentrate the virus solution, use small volume of Conservation buffer. Store the complexes at -80°C. NOTE: To reduce freezing/thawing cycles, it is recommended to aliquot virus for long term storage.
8. **Storage after elution**: Conservation Buffer can be added right after the elution step.
   a) Add the **Elution Buffer** to the virus/magnetic beads complexes pellet. To determine the appropriate volume of Elution Buffer to add, please see the table below. After adding Elution Buffer, incubate for 5 to 10 min at RT. Place the tube on the magnetic separation rack and incubate 10 to 30 min at RT. Adjust incubation time on the magnetic separation rack according to the volume. For tube volumes of 1 ml, 10 ml, 50 ml incubate the complexes on the magnetic rack for 15 min, 20 min and 30 min respectively.
   b) After Elution with the elution buffer, **save the supernatant** containing viruses and discard the pellet of magnetic beads.
   c) Add Add **Conservation Buffer (5x)** directly to the eluted viruses solution to obtain a 1X final concentration. For different fold concentrations please see the table below.
   d) Store virus at -80°C.

Table 2. Volume of Elution Buffer (EB) and Conservation Buffer (CB) for concentration and storage

Starting Viral Solution	Expected concentration 10X		Expected concentration 50X		Expected concentration 100X		Exchange Medium	
	EB	CB	EB	CB	EB	CB	EB	CB
1 ml	80 µl	20 µl	16 µl	4 µl	8 µl	2 µl	800 µl	200 µl
5 ml	400 µl	100 µl	80 µl	20 µl	40 µl	10 µl	4 ml	1 ml
10 ml	800 µl	200 µl	160 µl	40 µl	80 µl	20 µl	8 ml	2 ml
50 ml	4 ml	1 ml	800 µl	200 µl	400 µl	100 µl	40 ml	10 ml

X. Experimental Data

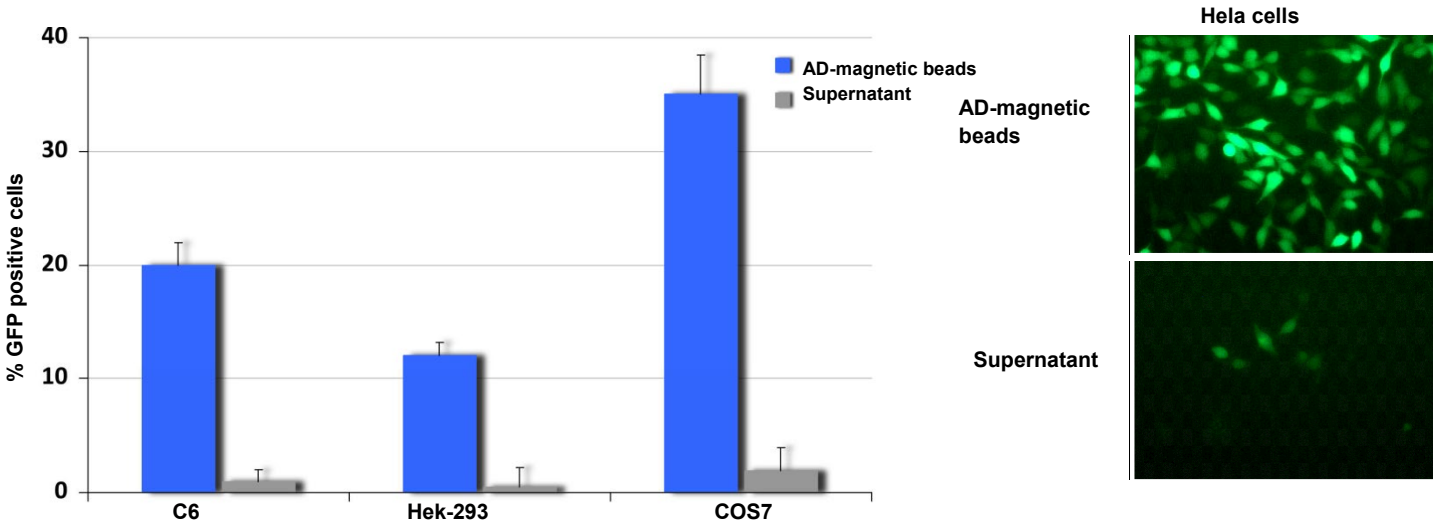

Fig.2. Efficiency of viral capture. Virus solution containing 10^7 viral particles/ml of adenovirus type 5 (dE1/E3) was mixed with AD-magnetic beads and captured by the magnetic field. Both supernatant (SN) and re-suspended magnetized virus were tested for infectivity on HeLa, C6, HEK-293 and COS7 cells. Cells were analyzed 24h post-transduction and the number of GFP positive cells (%) was monitored by FACS. The AD-magnetic beads efficiently captured adenoviruses (blue bars), the supernatants (grey bars) are nearly non-infectious (absence of virus) whereas the concentrated virus bound to the beads are highly infectious.

XI. General Troubleshooting Guide:

Problems	Suggestions
Low capture and concentration efficiency	1. Virus titer. Ensure that virus titer used corresponds to infectious viral particles and not physical particles. A 1 to 1000 ratio can be found between physical and infectious particles. 2. Virus quality and Medium purity. Even though the kit has been designed to work with culture medium containing viral particles, for the best efficiency, viruses should be as pure as possible, without contaminants. Endotoxins or microorganisms will easily bind on beads and compete with virus fixation. 3. Medium Composition. Use higher volume of magnetic beads to raise the capture efficiency. If medium is too viscous, dilute with PBS (with Ca^{2+} and Mg^{2+}) or with salt balanced physiological buffer at least 1.5 times. If using a complex medium (organic fluid…), dilute sample with PBS at least 1.5 times. Do not dilute medium excessively to avoid large dispersion of viral particles that hamper efficient virus capture. 4. Capture time. 15 min should give an optimal capture. It may be necessary to incubate magnetic beads longer with virus to ensure complete capture. 5. Reagents temperature. Reagents should have an ambient temperature and be vortexed prior to use.
Low elution efficiency	1. Incubation time. 10 min in the presence of Elution Buffer and 30 min on the separation rack should lead to optimal elution. It may be necessary to increase both incubation periods to 20 min and 45 min respectively. 2. Temperature. Perform the elution process at 37°C. 3. Washing. Before elution procedure, wash magnetic complexes with PBS as suggested in step 4.
Low infection efficiency	1. Cell density. A non-optimal density can lead to poor efficiency. The optimal confluency should range from 50 to 90% (true confluency, corresponding to 90% visual confluency) but most favorable density may vary according to the cell type. 2. Type of virus. Ensure that the virus can infect (being expressed) the cells. Another viral-driven promoter can be used as a control. 3. Cell condition. Use freshly thawed cells that have been passaged at least once. Cells should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) can alter the transduction efficiency. 4. Incubation time and transduction volume. 1) The optimal time range between transduction and assay varies with cells, promoter, expression product, etc. The transduction efficiency can be monitored after 24 – 96h by analyzing the gene product. Several reporter genes can be used to quantitatively monitor gene expression kinetics. 2) To increase transduction efficiency, transfection volume can be reduced for the first 24 hours. 5. Transgene detection assay. Ensure that your post-transduction assay is properly set up and includes a positive control. 6. Multiplicity of Infection (MOI). Be sure that the MOI is properly calculated. There could be a 1/10 to 1/1000 ratio between physical and infectious particles. 7. shRNA design. The design of an efficient shRNA is crucial. Ensure to use a validated shRNA

	sequence encoded in the expression vector.
Cellular toxicity	1. Unhealthy cells. 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc.), 4) Cells are too confluent or cell density is too low, 5) Verify equipment and materials. 2. Infection is toxic. Be sure that the cell line doesn't express the missing region for replication. It should be noted that even if virus can't replicate into the cells, it can still express viral proteins that can be toxic and cause cytopathic effect. Oncolytic viruses kill cells. 3. Concentration of magnetic/virus complexes too high. Decrease the amount of complexes added to the cells by lowering the MOI. 4. Incubation time. Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4 h to 24 h. 5. Key gene silencing. If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the si- or shRNA, this can lead to cell death.

XII. Related Products:

BioVision Product Name	Cat. No.	Size
Mag-Lentivirus and Retrovirus Purification Kit	K1458	-20, -100 Preps
Adenovirus Mini Purification Kit	K1300	-10, -20 Preps
Adenovirus Maxi Purification Kit	K1301	-2, -3, -10 Preps
Lentivirus Mini Purification Kit	K1305	-10, -20 Preps
Retrovirus Mini Purification Kit	K1307	-10, -20 Preps
Viral DNA extraction kit	K1446	-100 Preps
Magnetic Separator (Re-usable)	1999	1 separator
PEG Virus Precipitation Kit	K904	-50, -200 preps

FOR RESEARCH USE ONLY! Not to be used on humans.