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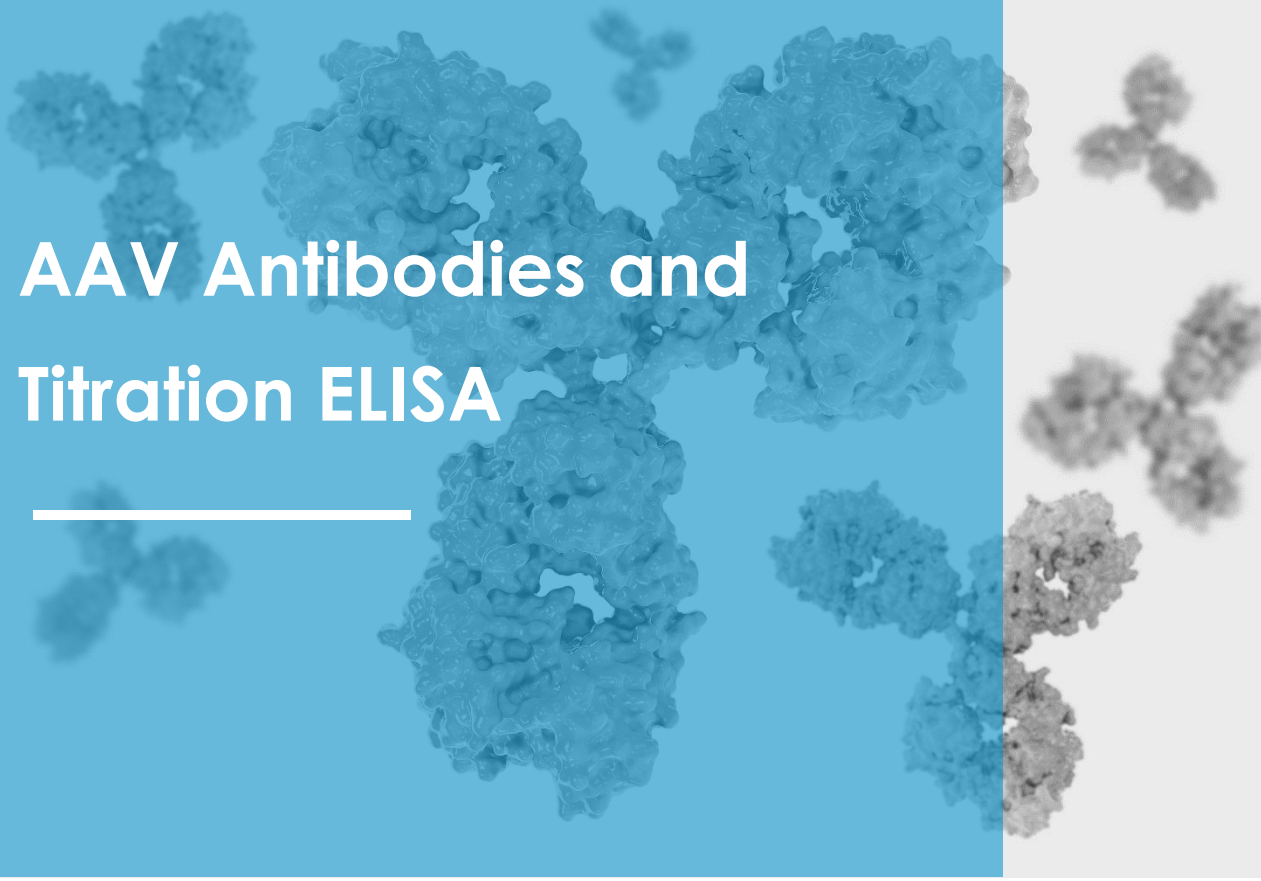
AAV Antibodies and Titration ELISA



1-631-624-4882 (USA) 44-161-818-6441 (Europe)

1-631-938-8221

info@creative-diagnostics.com

45-1 Ramsey Road, Shirley, NY 11967, USA

Introduction of AAV

Viral gene delivery systems include vectors developed from retrovirus (RV), adenovirus (AdV), adeno-associated virus (AAV), lentivirus (LV), and herpes simplex virus (HSV). AAV belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. There are eight different AAV serotypes reported to date. Adeno-associated virus (AAV) can infect humans and other primates, the infection of the virus can only cause a very slight immune response in the body. Interesting, gene therapy vectors using AAV can infect dividing and resting cells and persist in the extrachromosomal state without integration into the genome. These features make AAV to become an attractive candidate for creating gene therapy viral vectors and creating isogenic human disease models.

AAV Genome Structure

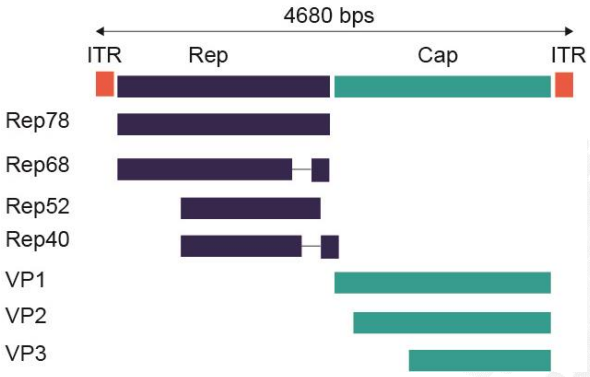


Figure 1. AAV2 Genome Map.

AAV has a linear single-stranded DNA (ssDNA) genome of approximately 4.7-kilobases (kb), with two 145 nucleotide-long inverted terminal repeats (ITR) at the termini. The virus does not encode a polymerase and therefore relies on cellular polymerases for genome replication. The ITRs flank the two viral genes rep (replication) and cap (capsid), encoding non-structural and structural proteins, respectively. The cap gene, through alternative splicing and initiation of translation, gives rise to three capsid proteins, VP1 (virion protein 1), VP2 and VP3, respectively. These capsid proteins assemble into a near-spherical protein shell of 60 subunits. The rep gene, through the use of two promoters and alternative splicing, encodes four regulatory proteins that are dubbed Rep78, Rep68, Rep52 and Rep40. These proteins are involved in AAV genome replication.

Due to many characteristics, wild-type AAV has attracted great interest from gene therapy researchers. The most important of these is the apparent lack of pathogenicity of the virus. It can also infect non-dividing cells and has the ability to stably integrate into a specific site in human chromosome 19 (referred to as AAVS1). This feature makes it more predictable than retroviruses with random insertion and mutagenic threats. AAV sequence is highly frequently integrated into specific loci, and the frequency of random incorporation into the genome is negligible. Interestingly, AAV, a vector for gene therapy, can eliminate this ability to integrate by removing rep and cap sequences from the DNA of the vector. After converting the single-stranded vector DNA into a double strand by the host cell DNA polymerase complex, a desired gene and a promoter driving the transcription of the gene are inserted between the inverted terminal repeats (ITR), the inverted terminal repeat helps form a concatemer in the nucleus. In non-dividing cells, these concatamers remain intact during the life of the host cell.

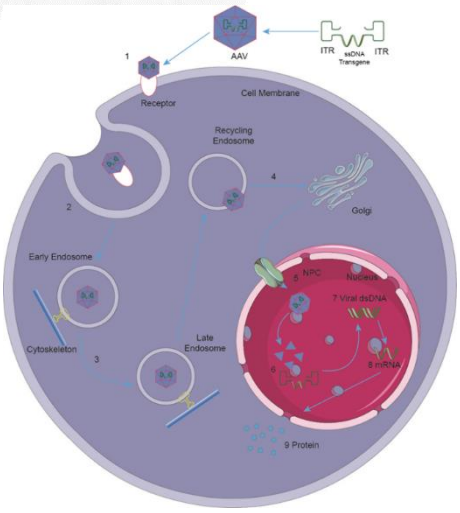


Figure 2. Steps of recombinant adeno-associated virus (rAAV) transduction.

AAV Serotypes

Adeno-associated virus (AAV) is an ssDNA virus that is a topic of intense study in gene therapy. The different serotypes transduce a wide variety of dividing and non-dividing cells showing long-term gene expression. The study found that AAV has multiple serotypes. The main difference between AAV vectors of different serotypes is that the capsid proteins are different. Currently, the most common AAV serotypes in gene delivery are AAV1, AAV2, AAV5, AAV6, AAV8, and AAV9.

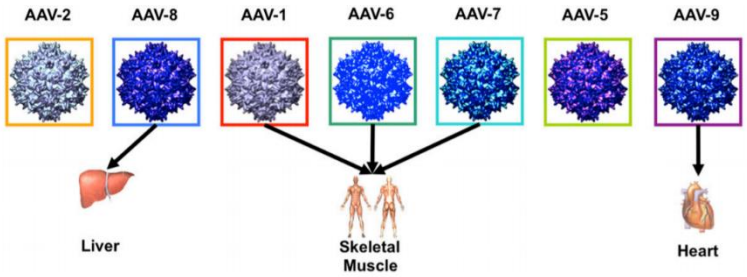


Figure 3. Partial-representation of the most commonly tested AAV vectors for gene transfer in small and large animal models and their main target tissues.

Studies find that the transfection efficiency is difference in different cell and tissure. Therefore, proper selection of appropriate AAV serotypes is essential.

AAV1

AAV1 has been used in several studies showing superior infection of muscle, liver and vascular endothelium when compared to other AAV serotypes. Until now no serious vector-related adverse effects have been observed. Methods for the characterization of AAV preparations currently include titration ELISA, real-time PCR, DNA dot blot, determination of transducing units, infectious center assay, SDSPAGE or electron microscopy. Immunotitration by AAV 1 Titration ELISA offers a fast, sensitive and reproducible method for titration of intact AAV1 wt virions, AAV1 recombinant virions or assembled and intact empty AAV 1 capsids.

AAV2

Recombinant AAV-2 is the most common serotype used in gene delivery. AAV2 presents natural tropism towards skeletal muscles, neurons, vascular smooth muscle cells and hepatocytes. AAV2 became the preeminent vector in translational programs for a multitude of reasons, not the least of which was that it was the first to be commercially produced at scale in support of clinical gene therapy programs. This early developmental lead created a powerful precedent that rendered AAV2 the frontrunner for clinical development, despite its comparatively low transduction efficiency.

AAV5

In addition, studies have found that recombinant AAV5 is more efficient than AAV2 in vivo to transfer b-galactosidase cDNA into mouse airways and alveolar epithelial cells, and that the mediated gene transfer is not inhibited by soluble heparin.

AAV6

Transduction efficiency was primarily dependent on the source of Cap protein, defined here as the vector pseudotype. The AAV6 and AAV2 pseudotype vectors exhibited different tropisms in tissue-cultured cells, and cell transduction by AAV6 vectors was not inhibited by heparin, nor did they compete for entry in a transduction assay, indicating that AAV6 and AAV2 capsid bind different receptors. In vivo analysis of vectors showed that AAV2 pseudotype vectors gave high transduction rates in alveolar cells but much lower rates in the airway epithelium. In contrast, the AAV6 pseudotype vectors exhibited much more efficient transduction of epithelial cells in large and small airways, showing up to 80% transduction in some airways. These results, combined with previous studies showing lower immunogenicity of AAV6 than of AAV2 vectors, indicate that AAV6 vectors may provide significant advantages over AAV2 for gene therapy of lung diseases like cystic fibrosis.

AAV8

AAV serotype 8 (AAV8) shows a significantly greater liver transduction efficiency than those of other serotypes, which has resulted in efforts to develop this virus as a gene therapy vector for hemophilia A and familial hypercholesterolemia. In addition, a comparison of the AAV8 and AAV2 capsid surface amino acids showed a reduced distribution of basic charge for AAV8 at the mapped AAV2 heparin sulfate receptor binding region, consistent with an observed non-heparin-binding phenotype for AAV8. Thus, this AAV8 structure provides an additional platform for mutagenesis efforts to characterize AAV capsid regions responsible for differential cellular tropism, transduction, and antigenicity for these promising gene therapy vectors.

AAV9

Isolated originally by James Wilson and colleagues, AAV9 is one of a very large family of AAV clades containing more than 100 new serotypes. In terms of the treatment of neurological diseases, a central reason for the emerging popularity of AAV9 is its remarkable ability to cross the BBB. The prospect of delivering therapeutic genes directly to the brain through the vasculature promises a more straightforward approach to treating a number of neurological diseases, particularly genetic diseases in infants that affect the structure. Because AAV9 also efficiently transduces both spinal motor neurons and dorsal root ganglia following systemic delivery, this vector may merit accelerated clinical development for diseases also affecting the structure and function of the spinal cord, such as amyotrophic lateral sclerosis, neuropathic pain, spinal injury, and certain ataxias.

Table 1. AAV serotypes and their tropism

AAV Serotype	Muscle	Liver	Lung	Brain	Retina	Pancreas	Kidney	Heart
AAV1	✓			Neurons and glial cells	✓	✓		✓
AAV2		✓			✓		✓	
AAV3		✓			✓			✓
AAV4				✓				✓
AAV5			Lung alveolar cells	Neurons and glial cells	✓			
AAV6	✓		✓					✓
AAV7	✓			Neurons	✓			
AAV8	✓	✓		Neurons	✓	✓		
AAV9	✓	✓	✓	Neurons	✓	✓	✓	✓

Table 2. Human Primary Cell Lines and AAV Serotypes

Adapted from Ellis et al 2013, Virology Journal. Cells were analyzed using flow cytometry at 48h post-infection. Legend shows the percentage of GFP positive cells. Legend: -, 0-5%; +, 5-40%; ++, 40-80%; +++, >80%.

AAV Serotype	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
BJ Fibroblasts	+	+	+	-	-	+	-	-	+
BJ hTERT Fibroblasts	++	+	+	-	-	+	-	-	-
ES Cell	-	+	++	-	-	+	-	-	-
HUVEC	+++	+++	++	-	+	+++	+	+	+
Keratinocyte	++	+	+	-	-	+++	-	-	-
Hematopoietic Progenitor	-	-	-	-	-	+	-	-	-

AAV Quality Assay

In recent, methods for the characterization of AAV preparations currently include titration ELISA, qPCR, ddPCR, DNA dot blot, determination of transducing units, infectious center assay, SDS-PAGE or electron microscopy. Among them, immunotitration by CD's AAV titration ELISA offers a fast, sensitive and reproducible method. The assay is based on the sandwich ELISA technique. A monoclonal antibody specific for a conformational epitope on assembled different AAV capsids is coated onto microtiter strips and is used to capture different AAV particles from the specimen. Captured AAV particles are detected in two steps. First a biotin conjugated monoclonal antibody to AAV is bound to the immune complex. In the second step streptavidin peroxidase conjugate reacts with the biotin molecules. Addition of substrate solution results in a color reaction which is proportional to the amount of specifically bound viral particles. The absorbance is measured photometrically at 450 nm. The kit control provided contains an AAV particle preparation of intact capsids. It shows a typical titration curve when used in dilutions of steps of two. It allows quantitative determination of samples of an unknown particle titer (immunological titer) and the calibration of an inhouse AAV preparation.

Table 3. The Features of Adeno-Associated Virus (AAV) Related Antibodies

Cat.No	Antigen	Serotype												Isotype	Application
		AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9	AAV10	AAV11	AAV12		
DMAB6350/ DMAB6351	Capsid	+	-	-	-	-	+	-	-	-	-	-	+	IgG2a	Dot, ELISA, ICC/IF, IHC-Fr, IHC-P, IP, Neut
CABT-B9061/ CABT-B9062	Capsid	-	+	+	-	-	-	-	-	-	-	-	-	IgG3	ELISA, ICC/IF, IHC-Fr, IHC-P, IP, Neut
DMAB6352/ CABT-B9063	Capsid	-	-	-	+	-	-	-	-	-	-	-	-	IgG2a	ELISA, ICC/IF, IHC-Fr, IHC-P, IP, Neut
DMAB6353/ DMAB6355	Capsid	-	-	-	-	+	-	-	-	-	-	-	-	IgG2a	Affinity Chromatography, ELISA, ICC/IF, IHC, IP
DMAB6354	Capsid	-	-	-	-	+	-	-	-	-	-	-	-	IgG2b	ELISA, ICC/IF, IHC-Fr, IHC-P, IP, Neut
CABT-B9064	Capsid	-	-	-	-	-	+	-	-	-	-	-	-	IgG2a	Dot, ELISA, ICC/IF, IHC-Fr, IHC-P, Neut
CABT-B9065	Capsid	+	-	+	-	-	-	+	+	-	+	-	-	IgG2a	Dot, ELISA, ICC/IF, IHC-Fr, IHC-P, IP, Neut
DPAB-AV01	Capsid	-	-	-	-	-	-	-	-	+	-	-	-	IgA	Dot, ELISA, ICC/IF, IHC-Fr, IHC-P, Neut
DMAB6348	AAV VP1	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	IgG2a	ELISA, ICC/IF, IP, WB
DMAB6349	AAV VP1/VP2	+	+	+	nd	nd	+	nd	nd	nd	nd	nd	nd	IgG1	ICC/IF,IHC, IP, WB
DPAB2423	AAV VP1/VP2/VP3	+	+	+	+	+	+	+	+	+	+	+	+	polyclonal	ELISA, ICC/IF, IP, WB
DMAB6345	AAV replicase	+	+	+	+	+	+	nd	nd	nd	nd	nd	nd	IgG1	WB
DMAB6346	AAV replicase	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	IgG1	Affinity Chromatography, ICC/IF, IP, WB
DMAB6347	AAV replicase	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	IgG1	ICC/IF, IP

nd——Not Determined

Related Product

Adeno-Associated Virus (AAV) Titration ELISA

Cat. No.	Product Name	Cat. No.	Product Name
DEIA590	AAV1 Titration ELISA Kit	DEIAAV6	AAV6 Titration ELISA Kit
DEIA589	AAV2 Titration ELISA Kit	DEIAAV8	AAV8 Titration ELISA Kit
DEIA591	AAV5 Titration ELISA Kit	DEIAAV9	AAV9 Titration ELISA Kit

Adeno-Associated Virus (AAV) Related antibodies

Cat. No.	Product Name	Description
DMAB6351	Anti-AAV1 (intact particle) monoclonal antibody, Clone BEL2b [Biotin]	Specifically detects AAV1 intact virus particles, both empty and full capsids. DMAB6351 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. Not suitable for Immunoblotting. In immunofluorescence, a weak cross-reactivity was observed with AAV6. No reaction with AAV type 2, 3, 4, 5.
DMAB6350	Anti-AAV1 (intact particle) monoclonal antibody, Clone BEL2b	Specifically detects AAV1 intact virus particles, both empty and full capsids. DMAB6350 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. Not suitable for Immunoblotting. In immunofluorescence and dot plot analysis, a weak cross-reactivity was observed with AAV6 and AAV12. No reaction with other AAV types.
CABT-B9061	Anti-AAV2 (intact particle) monoclonal antibody, clone B31 [Biotin]	Specifically detects intact virus particles, both empty and full capsids. With AAV2 and AAV3 CABT-B9061 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. Not for WB.
CABT-B9062	Anti-AAV2 (intact particle) monoclonal antibody, clone B31	Specifically detects intact virus particles, both empty and full capsids. With AAV2 and AAV3 CABT-B9061 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. Not for WB.
DMAB6346	Anti-AAV2 Replicase monoclonal antibody, Clone A227.8	Recognizes AAV replicase proteins Rep 78, Rep 68, Rep 52 and Rep 40 in AAV-infected cells. In Immunoblotting prominant reaction with Rep 78 and Rep 68, only faint reaction with Rep 52 and Rep 40.

Cat. No.	Product Name	Description
DMAB6345	Anti-AAV2 Replicase monoclonal antibody, Clone A304.10	Recognizes AAV replicase proteins Rep 78, Rep 68, Rep 52 and Rep 40 in human AAV-2-infected cells.
DMAB6347	Anti-AAV2 Replicase monoclonal antibody, Clone A77.4	Recognizes AAV replicase proteins Rep78 and Rep52 in human AAV2-infected cells, no reaction with Rep68 and Rep40.
DMAB6348	Anti-AAV2 VP1 monoclonal antibody, Clone B2	DMAB6348 reacts with VP1 of AAV-2; weak cross-reaction with serotypes 1, 3, 4, 5, 6. In immunoprecipitation, an occasional reaction with a non-AAV-derived protein is found.
DMAB6349	Anti-AAV2 VP1/VP2 monoclonal antibody, Clone B610	Recognizes AAV-2 viral capsid proteins VP1 and VP2, which are highly enriched in the nucleus. Weak cross-reaction with serogroups 1, 3, and 6.
DPAB2423	Anti-AAV2 VP1/VP2/VP3 polyclonal antibody	Reacts with VP1, VP2 and VP3. Raised against recombinant AAV-2 capsid proteins.
CABT-B9063	Anti-AAV4 (intact particle) monoclonal antibody, clone BEL5 [Biotin]	Specifically detects AAV4 intact virus particles, both empty and full capsids. CABT-B9063 recognizes a conformational epitope of assembled capsids not present in denatured and native unassembled capsid proteins. No cross-reaction with other AAV serotypes.
DMAB6352	Anti-AAV4 (intact particle) monoclonal antibody, Clone BEL5	Specifically detects AAV4 intact virus particles, both empty and full capsids. DMAB6352 recognizes a conformational epitope of assembled capsids not present in denatured and native unassembled capsid proteins. No cross-reaction with other AAV serotypes.
DMAB6355	Anti-AAV5 (intact particle) monoclonal antibody, Clone BEL6b [Biotin]	Specifically detects intact virus particles, both empty and full capsids. DMAB6355 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. No cross-reaction with other AAV serotypes. The antibody is not suitable for Immunoblotting.
DMAB6353	Anti-AAV5 (intact particle) monoclonal antibody, Clone BEL6b	Specifically detects intact virus particles, both empty and full capsids. DMAB6353 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. No cross-reaction with other AAV serotypes. The antibody is not suitable for Immunoblotting.

Cat. No.	Product Name	Description
DMAB6354	Anti-AAV5 (intact particle) monoclonal antibody, Clone BEL6c	Specifically detects intact virus particles, both empty and full capsids. DMAB6353 recognizes a conformational epitope of assembled capsids, not present in denatured and native unassembled capsid proteins. No cross-reaction with other AAV serotypes. The antibody is not suitable for Immunoblotting.
DPAB2424	Anti-AAV5 polyclonal antiserum	Raised against recombinant AAV5 capsid proteins. The antibody reacts with virus capsid proteins VP1, VP2, VP3 of AAV5; no cross-reaction with AAV types 1 - 4.
CABT-B9064	Anti-AAV6 (intact particle) monoclonal antibody, clone BEL7	Useful for analysis of the AAV assembly process. CABT-B9064 specifically reacts with intact adeno-associated virus particles, empty and full capsids. Recognizes a conformational epitope of assembled capsids. Specific for AAV-6. No cross-reaction with AAV type 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, and 12 was observed. Not for use in WB.
CABT-B9065	Anti-AAV8 (intact particle) monoclonal antibody, clone BEL9	For characterization of different stages of infection and very useful for analysis of the AAV assembly process. CABT-B9065 specifically reacts with intact adeno-associated virus particles, empty and full capsids. Recognizes a conformational epitope of assembled capsids. Not for use in immunoblotting.
CABT-B9066	Anti-AAV8/9 (intact particle) monoclonal antibody, clone BEL9/0	For characterization of different stages of infection and very useful for analysis of the AAV assembly process. CABT-B9066 specifically reacts with intact adeno-associated virus particles, empty and full capsids. Recognizes a conformational epitope of assembled capsids. The antibody cannot be used for immunoblotting using denaturing conditions.
DPAB-AV01	Anti-AAV9 (intact particles) monoclonal antibody, clone BEL0	Useful for analysis of the AAV assembly process. DPAB-AV01 specifically reacts with intact adeno-associated virus particles, empty and full capsids. Recognizes a conformational epitope of assembled capsids. Not for use in WB.

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