A LEAP FORWARD

IN AAV RESEARCH & GENE THERAPY

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ELISAs

Reliable AAV titer quantification
Specific recognition of intact AAV particles
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Validated assays for standardized approaches

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AAV ANTIBODIES
WITH BROAD APPLICATION RANGE

Immunochemical AAV studies

Anti-AAV virus particle antibodies are suitable for the characterization of different stages of adeno-associated virus (AAV) infection and are very useful for the analysis of the AAV capsid assembly. The antibodies specifically recognize conformational epitopes in assembled capsids of different AAV serotypes. Hence, they exclusively react with intact AAV particles.

Viral capsid protein antibodies (VP) exclusively recognize AAV capsid proteins and are useful for immunolocalization studies of AAV capsid formation, or immunoprecipitation and western blot analysis of viral capsid proteins.

Anti-AAV replicase antibodies (Rep) react with selected replicase (Rep) proteins in human AAV-infected cells. Applications include immunolocalization or immunoblotting studies to investigate the correlation between Rep expression and the course of an infection.

Antigen*	Product Specifications	Cat. No.
AAV1	mouse mAb, clone ADK1a	610150
AAV2	mouse mAb, clone A20	61055
AAV2	mouse rAb, clone A20R	610298
AAV4	mouse mAb, clone ADK4	610147
AAV5	mouse mAb, clone ADK5a	610148
AAV5	mouse mAb, clone ADK5b	610149
AAV5	rabbit polyclonal Ab	610137
AAV6	mouse mAb, clone ADK6	610159
AAV8	mouse mAb, clone ADK8	610160
AAV8/9	mouse mAb, clone ADK8/9	651161
AAV9	mouse mAb, clone ADK9	610162
VP1	mouse mAb, clone A1	61056
VP1/VP2	mouse mAb, clone A69	61057
VP1/VP2/VP3	mouse mAb, clone B1	61058
VP1/VP2/VP3	rabbit polyclonal Ab	61084
AAV-Rep	mouse mAb, clone 259.5	61071
AAV-Rep	mouse mAb, clone 226.7	65172
AAV-Rep	mouse mAb, clone 303.9	61069
AAV-Rep	mouse mAb, clone 76.3	61073

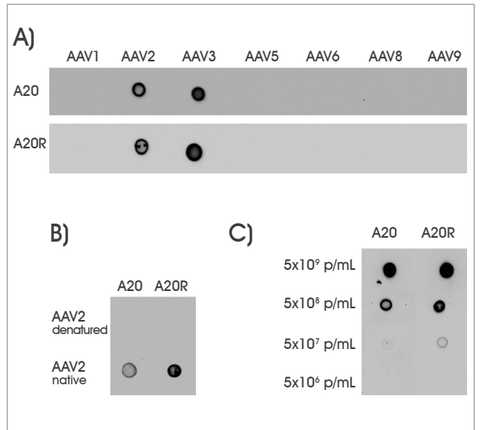
Ask us for antibodies against AAV serotypes 3, rh10, and 12.
Sample sizes and different formats at www.progen.com

AAV antibodies in gene therapy research

AAV vectors are powerful tools in gene therapy research and development. Recombinant AAV vectors (rAAV) corresponding to the different viral serotypes have successfully been used as universal gene shuttles in human cells.[9]

Neutralizing AAV antibodies present in serum or plasma may block transduction with AAV vectors. In a prescreening step before vector administration, the titer of neutralizing AAV antibodies can be determined in a cell-based assay. PROGEN's AAV antibodies are ideal positive controls that neutralize wild-type AAV capsids of AAV serotypes 1, 2, 5, 6, 8, and 9.[2-7]

PROGEN supplements its portfolio of AAV antibodies with advanced recombinant IgGs that feature optimal stability and batch-to-batch consistency. These antibodies retain equal performance compared to the mouse monoclonal antibodies with respect to cross-reactivity, AAV capsid recognition and binding sensitivity (see adjacent figure).



Comparison of PROGEN's A20 mouse monoclonal antibody with the recombinant A20R antibody (both against AAV2): A) Dot blot comparing cross-reactivities of A20 and A20R with different AAV serotypes. B) Dot blot analysis of antibody reactivity with denatured and native AAV2 capsids. C) Dot blot analysis of binding sensitivity of A20 and A20R by using decreasing AAV2 particle titers.

1 Naldini L, 2015, Nature, 526:351–360 (Review)
2 Bennett AD et al., 2018, Virology, 518:369–376
3 Tseng YS et al., 2015, J Virol, 89(3):1794–1808
4 Gurda BL et al., 2012, J Virol 86:7739–7751
5 Sonntag F et al., 2011, J Virol 85:12686–12697
6 Moskalenko M et al., 2000, J Virol, 74(4):1761–1766
7 Wobus CE et al., 2000, J Virol, 74(19):9281–9293

STANDARDIZED RESEARCH WITH
AAV TITRATION ELISAs

Reliable determination of AAV titers

The increasing interest in rAAV for clinical applications demands a dependable and reproducible quantification of accurate rAAV titers to ensure a safe and reliable gene transfer. In view of the scientific and clinical significance, PROGEN has established a line of AAV quantification ELISAs for different AAV serotypes (1, 2, 3, 5, 6, 8, 9, and rh10), utilizing its portfolio of capsid-specific AAV antibodies.

The assays for the determination of rAAV2 and rAAV8 titers have been validated in international studies[8, 9] and have been classified as superior method for a reliable, standardized rAAV particle titer quantification.

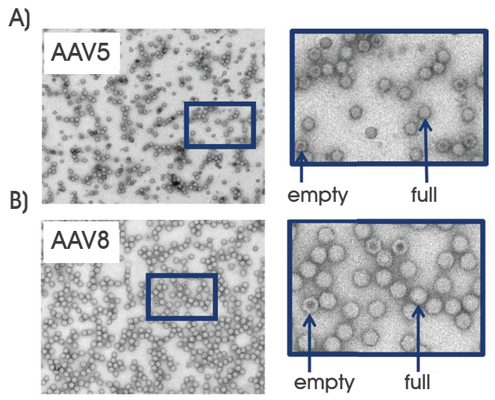
Serotype	ELISA Cat. No.
AAV1	PRAAV1
AAV2	PRATV
AAV2	PRAAV2R
AAV3	PRAAV3R
AAV5	PRAAV5
AAV6	PRAAV6
AAV8	PRAAV8
AAV9	PRAAV9
AAVrh10	PRAAV10 NEW!

Test our OptiPrep™ (No.114542) for damage-free rAAV purification!

8 Ayuso E et al., 2014, Human Gene Therapy 25:977–987
9 Lock M et al., 2010, Human Gene Therapy 21:1273–1285

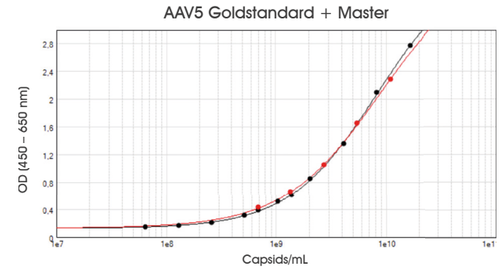
Development of internal AAV standards for ELISA kit calibration

The development of standardized AAV capsid material is an essential requirement for the calibration of all of PROGEN's AAV titration ELISA assays to ensure the reliable quantification of intact capsids in rAAV vector preparations. The alignment of the AAV2 and AAV8 Kit Controls for PROGEN's titration ELISAs is based on the ATCC standard material[8, 9]. For the remaining Kit Controls of the AAV1, 3, 5, 6 and 9 titration ELISAs PROGEN utilizes electron microscopy in combination with qPCR and ddPCR for the development of internal AAV gold standards (data for the development of the AAV5 standard shown below).



C)

	Product Specifications	Mean (qPCR-titer)	CV (qPCR)
A	AAV5 (3 labs, 16 runs)	7,1E+12 vg/mL	18%
B	rAAV2 RSM (ATCC)	3,8E+10 vg/mL	78%
C	rAAV8 RSM (ATCC)	9,6E+11 vg/mL	113%



A) + B) Comparison of AAV5 sample and reference AAV8 (WL217S) capsids & distinction of full and empty capsids by EM: A) AAV5 micrograph. B) AAV8 (WL217S) micrograph, which was used to characterize the current AAV8 RSM material from ATCC[8]. Samples were stained with uranyl acetate.

C) Comparison of AAV5 qPCR titers with ATCC standards for AAV2 and AAV8: A: 3 independent labs provided qPCR / ddPCR data for the AAV5 standard material. The mean titer of viral genome copies and CV was calculated. B: Published qPCR data for ATCC standard material AAV2[9]. C: Published qPCR data for ATCC standard material AAV8[8].

D) Alignment of the Kit Control with the established gold standard in the AAV5 Titration ELISA: Aligned curves of gold standard material and Kit Control, measured with the AAV5 Titration ELISA (OD vs. concentration). Black: Curve of the gold standard material, Red: Curve of the Kit Control.