Product datasheet

anti-AAV2 (intact particle) mouse monoclonal, A20, lyophilized, purified

Short overview

Cat. No.	61055
Quantity	50 µg
Concentration	50 µg/ml after reconstitution with 1 ml dist. water

Product description

Host	Mouse
Antibody Type	Monoclonal
Isotype	IgG3
Clone	A20
Immunogen	AAV2 capsids
Formulation	Lyophilized; reconstitute in 1 ml dist. water (final solution contains 0.09% sodium azide, 0.5% BSA in PBS buffer, pH 7.4)
Binding affinity	KD value (AAV2) = 2.6E-11 M KD value (AAV3) = <1.0E-12 M
Synomym	Adeno-associated virus 2; AAV-2
Conjugate	Unconjugated
Purification	Affinity chromatography
Storage before reconstitution	2-8°C until indicated expiry date
Storage after reconstitution	2-8°C
Intended use	Research use only
Application	Affinity chromatography, Dot blot, ELISA, ICC/IF, IP, Neutralization assay
Reactivity	AAV2, AAV2 7m8, AAV3
No reactivity	AAV1, AAV11, AAV12, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVDJ, AAVrh10, AAVrh74

Applications

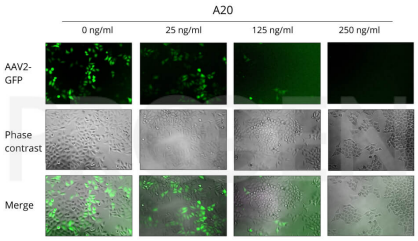
Affinity Chromatography	Assay dependent
Dot Blot	1:500 (0.1 µg/ml; non-denaturing conditions)
ELISA	Assay dependent
Immunocytochemistry (ICC)	1:20
Immunoprecipitation (IP)	1:5
Neutralization Assay	EC50 ~5 ng/ml (AAV2) and ~3 ng/ml (AAV3) - assay dependent

Background

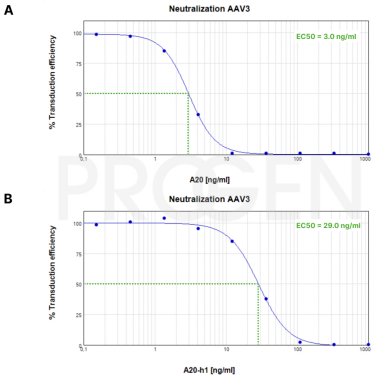
For characterization of different stages of infection and very useful for the analysis of the AAV2 assembly process. A20 specifically reacts with AAV2 and AAV3, empty and full capsids. Recognizes a conformational epitope of assembled capsids, not present in denatured capsid proteins and native but unassembled capsid proteins. The antibody cannot be used for immunoblotting.Epitope mapping experiments (Wobus et al. 2000) identified four immunoreactive (discontinous) regions. The major reaction was attributed to sequence aa 369 to aa378 of AAV2 capsids. The antibody is also useful for neutralizing experiments.

Wobus, C. E. et al. Monoclonal antibodies against the adeno-associated virus type 2 (AAV-2) capsid: epitope mapping and identification of capsid domains involved in AAV-2-cell interaction and neutralization of AAV-2 infection. J. Virol. 74, 9281-93 (2000). Moskalenko, M. et al. Epitope Mapping of Human Anti-Adeno-Associated Virus Type 2 Neutralizing Antibodies: Implications for Gene Therapy and Virus Structure. Jounal Virol. 74, 1761-1766 (2000).

Product images



Neutralization of AAV2-GFP vectors with the A20 antibody (Cat. No. 61055). AAV infection was shown in HeLa cells and photos (GFP, CPE, merge) were taken ~48 h post infection. Neutralization was enhanced with increasing A20 concentration.



Neutralization of AAV3 with mouse monoclonal AAV2 antibody clone A20 (A) and human chimeric AAV2 antibody clone A20-h1 (B) by using AAV3-NanoLuc® viral particles from Promega. (A) anti-AAV2 (intact particle) mouse monoclonal, A20 (Cat. No. 61055) or (B) anti-AAV2, human chimeric, A20-h1 (Cat. No. 692379) were preincubated with AAV3-NanoLuc® viral particles for 30 min at RT at 300 rpm (antibody concentrations 0.2-3,000 ng/ml). HEK293 cells (100 µl) were plated at 200,000 cells/ml in DMEM + 1% FCS. Virus-antibody-mix (20 µl) was added to the cells and incubated for 16-24 h at 37°C. Extracellular NanoLuc Inhibitor and Nano-Glo® Live Cell Assay System (Promega) was added to the wells and incubated for 5 min at RT at 300 rpm. Luminescence was measured using an ID5-Reader and plotted with Softmax Pro 7.1 software to determine the EC50 values.

Serotype	Clone	Method	Residues						Reference
			200 - 299	300 - 399	400 - 499	500 - 599	600 - 699	700 - 731	
AAV2	A20	Cryo-EM	253, 254, 258, 261, 262, 264	384, 385		548, 556	658 - 660	708, 717	1
		Peptide scanning	272 - 281	369 - 378		560 - 573			2
		Peptide insertion	261	381		534, 573			2, 3
		Site-directed mutagenesis	263, 264	384, 385		548		708	4

[1] McCraw et al. Structure of adeno-associated virus-2 in complex with neutralizing monoclonal antibody A20. Virology (2012) **431**:40–9.
[2] Wobus et al. Monoclonal antibodies against the adeno-associated virus type 2 (AAV-2) capsid: epitope mapping and identification of capsid domains involved in AAV-2-cell interaction and neutralization of AAV-2 infection. J Virol (2000) **74**:9281–93.
[3] Huttner et al. Genetic modifications of the adeno-associated virus type 2 capsid reduce the affinity and the neutralizing effects of human serum antibodies. Gene Ther (2003) **10**:2139–47.
[4] Lochrie et al. Mutations on the external surfaces of adeno-associated virus type 2 capsids that affect transduction and neutralization. J Virol (2006) **80**:821–34.

*Residues boxed in green have been identified with at least two independent methods.

Several publications cited below describe the analysis of binding sites for the A20 antibody using different techniques. Multiple amino acids have been identified, that are very likely to be part of the binding site, especially those that were identified with more than one method (green boxes).

The amino acids of each binding site are located in different parts of the protein chains and are recognized as the epitope of the antibody only in the assembled capsid where they are in close proximity to each other and in the correct conformation. Note that they do not react with denaturated AAV2-VP.

1 McCraw et al. Structureof adeno-associated virus-2 in complex with neutralizing monoclonal antibody A20. Virology (2012) 431:40-9.

2 Wobus, C. E. et al. Monoclonal antibodies against the adeno-associated virus type 2 (AAV-2) capsid: epitope mapping and identification of capsid domains involved in AAV-2-cell interaction and neutralization of AAV-2 infection. J. Virol. 74, 928193 (2000).

3 Huttner et al. Genetic modifications of the adeno-associated virus type 2 capsid reduce the affinity and the neutralizing effects of human serum antibodies. Gene Ther (2003) 10:2139-47.

4 Lochrie et al. Mutations on the external surfaces of adeno-associated virus type 2 capsids that affect transduction and neutralization. J Virol. (2006) 80:821-34.

References

Publication	Species	Application
Ohba K. et al. Adeno-associated virus vector system controlling capsid expression improves viral quantity and quality., iScience, 26, 106487, (2023).	AAV2	IP
Haar, J., Blazevic, D., Strobel, B., Kreuz, S. & Michelfelder, S. MSD-based assays facilitate a rapid and quantitative serostatus profiling for the presence of anti-AAV antibodies. Mol. Ther. - Methods Clin. Dev. 25, 360â€“369 (2022).	AAV2	IA
Emmanuel, S. N., Mietzsch, M., Tseng, Y. S., Smith, J. K. & Agbandje-Mckenna, M. Parvovirus Capsid-Antibody Complex Structures Reveal Conservation of Antigenic Epitopes across the Family. Viral Immunol. 34, 3â€“17 (2021).	AAV2	binding region
Hamann, M. V. et al. Improved targeting of human CD4+ T cells by nanobody-modified AAV2 gene therapy vectors. PLoS One 16, (2021).	AAV2	dot blot
Bennett, A. et al. Structure comparison of the chimeric AAV2.7m8 vector with parental AAV2. J. Struct. Biol. 209, 107433 (2020).	AAV2,AAV2.7m8	dot blot