Product datasheet

anti-AAV5 (intact particle) mouse monoclonal, ADK5a, lyophilized, purified

Short overview

Cat. No.	610148
Quantity	50 µg
Concentration	50 µg/ml after reconstitution with 1 ml dist. water

Product description

Host	Mouse
Antibody Type	Monoclonal
Isotype	IgG2a kappa
Clone	ADK5a
Immunogen	AAV5 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 (AAV5) = 4.9E-11 M
Synomym	Adeno-associated Virus 5, AAV-5
Conjugate	Unconjugated
Purification	Affinity chromatography
Storage before reconstitution	2-8°C until indicated expiry date
Storage after reconstitution	Up to 3 months at 2-8°C; long term storage in aliquots at -20°C; avoid freeze/thaw cycles
Intended use	Research use only
Application	Affinity chromatography, Dot blot, ELISA, ICC/IF, IP, Neutralization assay
Reactivity	AAV5
No reactivity	AAV1, AAV11, AAV12, AAV2, AAV3, AAV4, 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 ~7 ng/ml (AAV5) - assay dependent

Background

For characterization of different stages of infection and very useful for the analysis of the AAV5 assembly process. ADK5a specifically reacts with intact adeno-associated virus 5 particles, 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.The antibody is usefull for neutralizing experiments.

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Product images

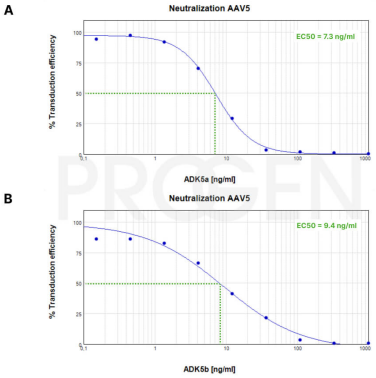
Serotype	Clone	Contact residues	Footprint residues	*VR
AAV5	ADK5a	244 N, 246 Q, 248-RE<u>IKSGSVD-256, 263 Y</u> <u>377 E, 378 N</u> <u>453 L, 456 R</u> <u>532 Q, ***533 P***, 535-NPGTTVPSATYL-543, 546 N</u> <u>653 V, 654 P, 656 S</u> <u>697 Q, 698 F, 704-DSTGEYR-710</u>	218, 240-<u>250-258, 261, 263</u>, 267, ***279*** 331, ***350***, 355-***359***-360, 364, 365, <u>377, 378</u>, 395 429-432, 437, <u>450, 451, 453-456</u>, 458, 459 <u>530-533-543, 545-548</u> 639, 641, <u>642, 648-***650, 651***, 653-656</u>-658, 660-662, <u>697-700, 704-712</u>	I III IV VII HI loop IX
	ADK5b	248 R <u>346-VQDS-319</u> <u>443 N</u> <u>530-NSQ5PAN-535, 540-ATYL-543, 545 G, 546 N</u> <u>697 Q, 704 D, 706 T, 708-EYR-710</u>	241-248 313-<u>315-319, 321, 323</u>, 355, 356, 358-***359-361, 362*** <u>440-443, 446-449</u> <u>530-***533***-548</u> <u>645-***650, 651***, 653-656-661</u> <u>697, 698, 704-712</u>	II IV VII HI loop IX

Tseng et al. Adeno-Associated Virus Serotype 1 (AAV1)- and AAV5-Antibody Complex Structures Reveal Evolutionary Commonalities in Parvovirus Antigenic Reactivity. Journal of Virology (2015) **89**:1794-1808.

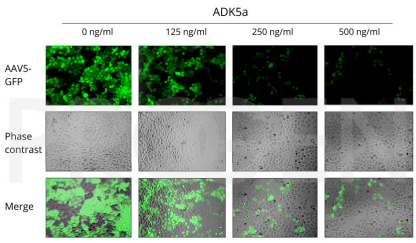
In the publication cited below multiple contact sites and footprint residues for ADK5a and ADK5b have been identified, that are very likely to be part of the binding site. The amino acids of each binding site are located in different parts of the protein chains and are recognized as the epitope of the antibody only in the assembled capsid where they are in close proximity to each other and in the correct conformation.

*The residues in the VR are underlined. Those involved in AAV5 receptor binding are bold and italicized.

Tseng et al. Adeno-Associated Virus Serotyp 1 (AAV1)- and AAV5-Antibody Complex Structures Reveal Evolutionary Commonalities in PArvovirus Antigenic Reactivity. J. Virol. 89:1794-1808 (2015).



Neutralization of AAV5 with mouse monoclonal AAV5 antibody clone ADK5a (A) and mouse monoclonal AAV5 antibody clone ADK5b (B) by using AAV5-NanoLuc® viral particles from Promega. (A) anti-AAV5 (intact particle) mouse monoclonal, ADK5a (Cat. No. 610148) or (B) anti-AAV5 (intact particle) mouse monoclonal, ADK5b (Cat. No. 610149) were preincubated with AAV5-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.



Neutralization of AAV5-GFP vectors with the ADK5a antibody (Cat. No. 610148). AAV infection was shown in HeLa cells and photos (GFP, CPE, merge) were taken ~48 h post infection. Neutralization was enhanced with increasing ADK5a concentration.

References

Publication	Species	Application
Silveria, M. A., Large, E. E., Zane, G. M., White, T. A. & Chapman, M. S. The structure of an aav5-aavr complex at 2.5 Ã… resolution: Implications for cellular entry and immune neutralization of aav gene therapy vectors. Viruses 12, (2020).	AAV5	neutralization
Earley, L. F. et al. Adeno-associated Virus (AAV) Assembly-Activating Protein Is Not an Essential Requirement for Capsid Assembly of AAV Serotypes 4, 5, and 11. J. Virol. 91, 1980â€“1996 (2017).	AAV5	ICC-IF
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).	AAV5	binding region
Jose, A. et al. High-Resolution Structural Characterization of a New Adeno-associated Virus Serotype 5 Antibody Epitope toward Engineering Antibody-Resistant Recombinant Gene Delivery Vectors. J. Virol. 93, 1394â€“1412 (2019).	AAV5	cryoEM
Ohba K. et al. Adeno-associated virus vector system controlling capsid expression improves viral quantity and quality., iScience, 26, 106487, (2023).	AAV5	IP