Product datasheet

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

Short overview

Cat. No.	610148S
Quantity	10 µg
Concentration	50 µg/ml after reconstitution with 200 µl PBS

Product description

Host	Mouse
Antibody Type	Monoclonal
Isotype	IgG2a kappa
Clone	ADK5a
Immunogen	AAV5 capsids
Formulation	Lyophilized; reconstitute in 200 µl sterile PBS
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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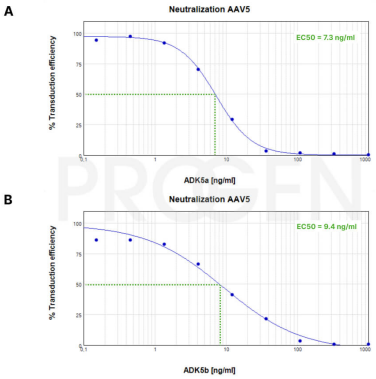
Product images



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.

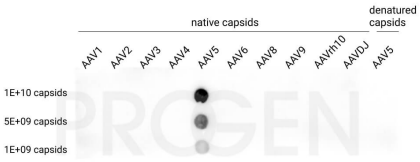
Serotype	Clone	Contact residues	Footprint residues	*VR
AAV5	ADK5a	244 N, 246 Q, 248-REIKSGSVD-256, 263 Y 377 E, 378 N 453 L, 456 R 532 Q, ***533 P***, 535-NPGTTVPSATYL-543, 546 N 653 V, 654 P, 656 S 697 Q, 698 F, 704-DSTGEYR-710	218, 240-250-258, 261, 263, 267, ***279*** 331, ***350***, 355-***359***-360,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



Dot blot analysis of native AAV1-AAV9, AAVrh10, AAVDJ capsids (1E+09-1E+10 capsids) and denatured AAV5 capsids (1E+09-1E+10 capsids, denatured at 95°C for 10 min in sample buffer).The nitrocellulose membrane was blocked with 5% dry milk in PBST (PBS + 0.1% Tween 20) for 1 h at RT. The primary antibody anti-AAV5 (intact particle) mouse monoclonal, ADK5a (Cat. No. 610148) was diluted in blocking buffer (antibody concentration 100 ng/ml) and incubated for 1 h at RT. The secondary antibody goat anti-mouse IgG HRP was also diluted in blocking buffer (antibody concentration 200 ng/ml) and incubated for 1 h at RT. The bands were visualized by chemiluminescent detection using PierceTM ECL Western Blotting Substrate.

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).	AAV5	IP
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
Baatartsogt, N. et al. A sensitive and reproducible cell-based assay via secNanoLuc to detect neutralizing antibody against adeno-associated virus vector capsid. Mol. Ther. - Methods Clin. Dev. 22, 162â€“171 (2021).	AAV5	Neutralization
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
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