

Product datasheet

anti-AAV5 (intact particle) mouse monoclonal, ADK5b, lyophilized, purified, sample

Short overview

Cat. No.	610149S
Quantity	10 µg
Concentration	50 µg/ml after reconstitution with 200 µl PBS

Product description

Host	Mouse
Antibody Type	Monoclonal
Isotype	IgG2b kappa
Clone	ADK5b
Immunogen	AAV5 capsids
Formulation	Lyophilized; reconstitute in 200 µl sterile PBS
Binding affinity	KD value (AAV5) = 6.0E-11 M
Synonym	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	Dot blot, ELISA, ICC/IF, IP, Neutralization assay
Reactivity	AAV5
No reactivity	AAV1, AAV2, AAV3, AAV4, AAV6, AAV8, AAV9, AAVDJ, AAVrh10, AAVrh74

Applications

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 ~9 ng/ml (AAV5) - assay dependent

Background

For characterization of different stages of infection and very useful for the analysis of the AAV5 assembly process. ADK5b 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 useful

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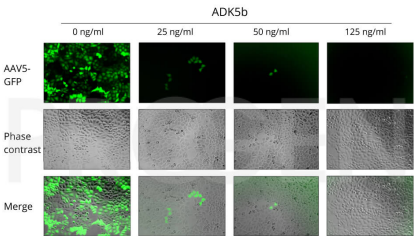
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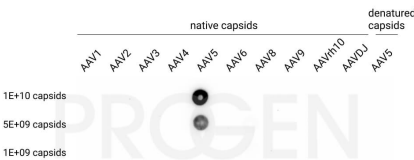
for neutralizing experiments.

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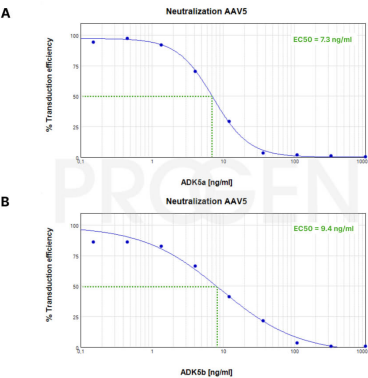
Product images



Neutralization of AAV5-GFP vectors with the ADK5b antibody (Cat. No. 610149). AAV infection was shown in HeLa cells and photos (GFP, CPE, merge) were taken ~48 h post infection. Neutralization was enhanced with increasing ADK5b concentration.



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, ADK5b (Cat. No. 610149) 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 Pierce™ ECL Plus Western Blotting Substrate.



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.

References

Publication	Species	Application
Emmanuel, S. N. et al. Structurally Mapping Antigenic Epitopes of Adeno-associated Virus 9: Development of Antibody Escape Variants. J. Virol. 96, (2022).	AAV5	dot blot
Kasprzyk, T., Triffault, S., Long, B. R., Zoog, S. J. & Vettermann, C. Confirmatory detection of neutralizing antibodies to AAV gene therapy using a cell-based transduction inhibition assay. Mol. Ther. - Methods Clin. Dev. 24, 222â€“229 (2022).	AAV5	TI assay
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
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 Å... 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, dot blot