Product datasheet

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

Short overview

Cat. No.	610149
Quantity	50 µg
Concentration	50 µg/ml after reconstitution with 1 ml dist. water

Product description

Host	Mouse
Antibody Type	Monoclonal
Isotype	IgG2b kappa
Clone	ADK5b
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) = 6.0E-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	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 usefull for neutralizing experiments.

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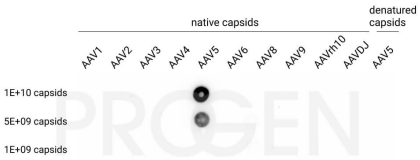
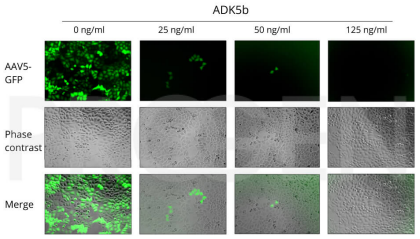
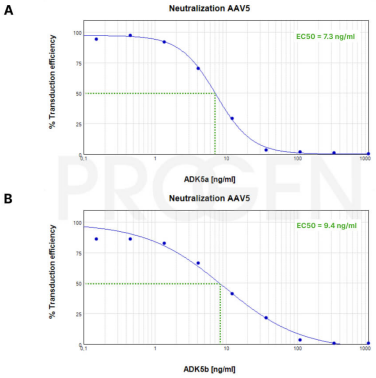
Product images



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 PierceTM ECL Plus Western Blotting Substrate.



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.



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 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, dot blot