Product datasheet

AAV5 VP3, recombinant protein

Short overview

Cat. No.	640835
Quantity	10 µg
Concentration	100 µg/ml (1.61 µM)

Product description

Formulation	Liquid, 6 M urea in PBS
Source	Escherichia coli
Molecular Weight	61.7 kDa (calculated Mw from aa sequence)
Purity	> 90% (determined by SDS PAGE)
Product description	N-terminal His-tagged (MGSSHHHHHHSSGLVPRGSH) recombinant AAV5 capsid protein VP3
Purification	Ni-NTA chromatography
Storage	-80°C
Intended use	Research use only
Application	Dot blot, SDS PAGE, WB

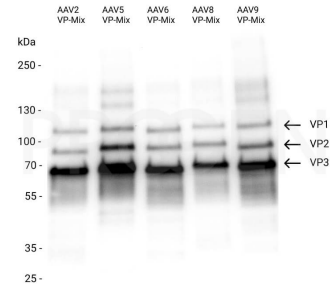
Applications

Dot Blot	100 ng, depending on primary antibody and detection method
SDS PAGE	1 µg
Western Blot (WB)	5-20 ng, depending on primary antibody and detection method

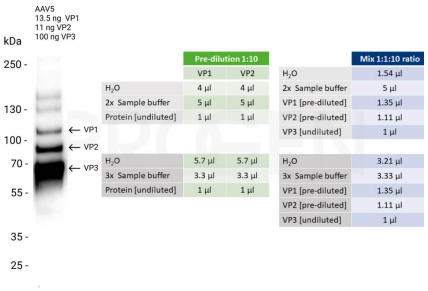
Background

The AAV capsid consists of three capsid proteins, i.e. VP1, VP2 and VP3, which differ in their N-terminus and encapsulate the genomic ssDNA. In native virus particles, the three proteins form subunits with a ratio of 1:1:10 (VP1:VP2:VP3), in a total number of 60 subunits per capsid. The recombinant AAV5 VP3 protein in combination with recombinant AAV5 VP1 (Cat. No. 640833) and recombinant AAV5 VP2 (Cat. No. 640834) can be used to create a mixture with the precise molar ratio of 1:1:10 to compare the protein composition of the viral capsid in your sample by protein detection methods, e.g. western blot. All three recombinant AAV5 capsid proteins are available as set (Cat. No. 72005) or as individual proteins (Cat. No. 640833, 640834, 640835).Note: please find an example how to prepare western blot samples in the pipetting scheme below. Aliquots of the remaining samples can be stored at -80°C for reuse.

Product images



Western blot analysis of recombinant AAV2 VP proteins (Cat. No. 72001), recombinant AAV5 VP proteins (Cat. No. 72005), recombinant AAV6 VP proteins (Cat. No. 72006), recombinant AAV8 VP proteins (Cat. No. 72008) and recombiant AAV9 VP proteins (Cat. No. 72009) with B1 antibody (Cat. No. 690058). Western blot analysis was performed on the precise molar ratio of 1:1:10 (13.5 ng VP1:11 ng VP2:100 ng VP3) combined in one lane. The PVDF membrane was blocked with 5% milk in PBST for 1 h at RT.The primary antibody anti-AAV VP1/VP2/VP3, B1 (Cat. No. 690058) was diluted in blocking buffer (antibody concentration 500 ng/ml) and incubated for 1 h at RT. The secondary antibody 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 Western Blotting Substrate.



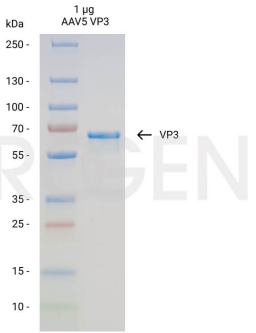
	Pre-dilution 1:10	
	VP1	VP2
H_2O	4 µl	4 µl
2x Sample buffer	5 µl	5 µl
Protein [undiluted]	1 µl	1 µl

H_2O	5.7 µl
3x Sample buffer	3.3 µl
Protein [undiluted]	1 µl

	Mix 1:1:10 ratio
H_2O	1.54 µl
2x Sample buffer	5 µl
VP1 [pre-diluted]	1.35 µl
VP2 [pre-diluted]	1.11 µl
VP3 [undiluted]	1 µl

H_2O	3.21 µl
3x Sample buffer	3.33 µl
VP1 [pre-diluted]	1.35 µl
VP2 [pre-diluted]	1.11 µl
VP3 [undiluted]	1 µl

Pipetting scheme for western blot analysis using a mix of the AAV5 capsid proteins (Cat. No. 640833, 640834, 640835). To create a VP mixture with the molar ratio 1:1:10 (VP1:VP2:VP3), please pre-dilute VP1 and VP2 1:10 to yield a final concentration of 10 µg/ml (green table). Pipette the pre-diluted VP1 and VP2 proteins and mix them with the undiluted VP3 protein in your sample buffer and water (blue table). The example with 2x and 3x sample buffer and the required volumes are indicated in the pipetting scheme. Thus, in one lane, 10 µl of the VP mix can be loaded onto the SDS PAGE and analyzed by Western blot using the B1 antibody (Cat. No. 690058, Cat. No. 61058-488, Cat. No. 61058-647).Undiluited = 100 µg/ml, pre-diluted = 10 µg/ml



SDS PAGE analysis to evaluate the purity of the AAV5 VP3 (Cat. No. 640835). To perform SDS PAGE analysis, 1 µg of protein was diluted in 10 µl PBS and sample buffer and denatured at 95°C for 5 min. The sample was loaded onto a 4-20% gradient gel (40 min at 200 V). Afterwards, the gel was stained for 1 h at RT with Coomassie solution and destained with water. The purity of AAV5 VP3 is > 90%.