Product datasheet

AAV5 VP1 + VP2 + VP3, recombinant proteins, set

Short overview

Cat. No.	72005
Quantity	10 µg each protein
Concentration	100 µg/ml (VP1: 1.19 µM, VP2: 1.45 µM, VP3: 1.61 µM)

Product description

Formulation	Liquid, 6 M urea in PBS
Source	Escherichia coli
Molecular Weight	VP1: 82.6 kDa, VP2: 67.7 kDa, VP3: 61.7 kDa (calculated Mw from aa sequence)
Purity	> 90% (determined by SDS PAGE)
Product description	N-terminal His-tagged (MGSSHHHHHHSSGLVPRGSH) recombinant AAV5 capsid proteins VP1 + VP2 + VP3
Purification	Ni-NTA chromatography
Storage	-80°C
Intended use	Research use only
Application	Dot blot, SDS PAGE, WB

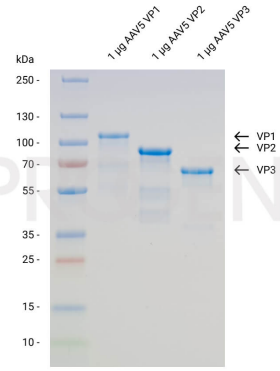
Applications

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

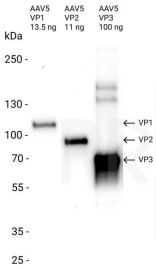
Background

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. This set of recombinant AAV5 VP1, VP2 and VP3 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. Set content:Cat. No. 640833 AAV5 VP1, recombinant protein Cat. No. 640834 AAV5 VP2, recombinant protein Cat. No. 640835 AAV5 VP3, recombinant protein

Product images

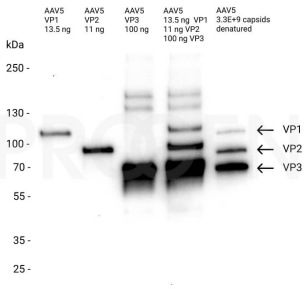


SDS PAGE analysis to evaluate the purity of the AAV5 VP1, VP2 and VP3 (Cat. No. 640833, 640834, 640835). To perform SDS PAGE analysis, 1 µg of protein was diluted in 10 µl PBS and sample buffer and denatured at 95°C for 5 min. The samples were loaded onto a 4-20% gradient gel (40 min at 200 V). Afterwards, the gel was stained for 1 h at RT with Coomassie solution and destained with water. The purity of AAV5 VP1, VP2 and VP3 is > 90%.



	VP1	VP2	VP3
H_2O	48.65 µl	48.89 µl	4 µl
2x Sample buffer	50 µl	50 µl	5 µl
Protein [100 µg/ml]	1.35 µl	1.11 µl	1 µl
H_2O	65.35 µl	65.59 µl	5.7 µl
3x Sample buffer	33.3 µl	33.3 µl	3.3 µl
Protein [100 µg/ml]	1.35 µl	1.11 µl	1 µl

Pipetting scheme for western blot analysis using the AAV5 capsid proteins (Cat. No. 640833, 640834, 640835) in separate lanes. To analyze the molar ratio of 1:1:10, it is recommended to load VP1, VP2 and VP3 as described in the pipetting scheme above. Therefore, the indicated volumes of the proteins (concentration 100 µg/ml) should be diluted with the appropriate amount of sample buffer and distilled water. 10 µl of each solution can be separately 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).



Western blot analysis of recombinant AAV5 capsid proteins (Cat. No. 640833, 640834, 640835) and denatured AAV5 capsids with B1 antibody (Cat. No. 690058). Western blot analysis was performed on the precise molar ratio of 1:1:10 (VP1:VP2:VP3) either in separate lanes or combined in one lane and on 3.3E+09 denatured AAV5 capsids. 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.