Product datasheet

AAVrh74 standard material (eGFP)

Short overview

Cat. No.	66V741
Quantity	100 µl
Concentration	> 1.0E+12 vg/ml; please find the lot-specific concentration (total capsid and viral genome titer) on the CoA

Product description

Filling grade	> 70% full capsids, please find the lot-specific filling grade on the CoA
Formulation	PBS + 0.014% Tween20 + 1 mM MgCl2 + 2.5 mM KCl
Source	Produced in HEK293T cells
Purity	> 95% by SDS-PAGE using Stain-Free Technology (Bio-Rad)
Quality check	Total capsid titer was assigned using AAVrh10 ELISA kits (PRAAV10) and the AAVrh74 kit control (PRAAV74-C) and viral genome titer by qPCR using eGFP primers; QC included analysis of filling grade, purity, aggregation, and endotoxin testing.
Transfer Vector	pAAV-CMV-eGFP-WPRE
Packaging Plasmid	pRep2-Caprh74 + pHelper
Endotoxin	< 1.0 EU/ml (detection limit 1.0 EU/ml)
Note	Please centrifuge before opening to ensure complete recovery of vial contents; aliquoting and repeated freeze/thaw cycles can lead to a drop in titer
Purification	Affinity Chromatography (POROS CaptureSelect AAVX Affinity Resin, Thermo Fisher Scientific), Iodixanol gradient centrifugation for removal of empty capsids using OptiPrep (Cat. No. 1893, PROGEN)
Storage	Up to 2 weeks: 2-8°C; long term storage in aliquots at -80°C; avoid > 5 freeze/thaw cycles
Intended use	Research use only
Application	Calibration of instruments e.g. mass photometry, Cell-based assay, Dot blot, ELISA, WB, ddPCR, qPCR

Applications

Dot Blot	Depending on primary antibody and detection method
ELISA	As a positive control in ELISA, dilute in ASSB 1x (provided with PROGENs AAV ELISA), analysis at least in duplicates is recommended
Cell-based Assay	Depending on the experimental setup
PCR	As standard or positive control in qPCR or ddPCR - concentration depending on experimental setup
Western Blot (WB)	Depending on primary antibody and detection method

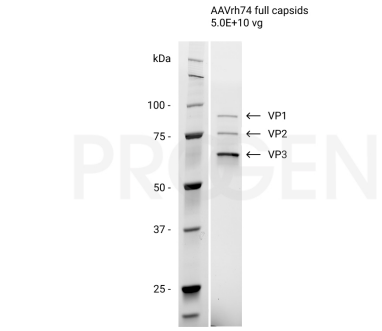
Background

Our AAVrh74 standard material consists of fully assembled AAVrh74 capsids with an eGFP reporter gene controlled by a CMV promotor. Final

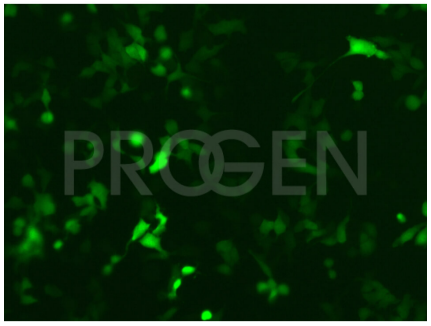
concentrations (total capsids and viral genome titer) are lot-specific and can be found on the CoA.
The AAVrh74 standard material is provided with a titer above 1.0E+12 viral genomes/ml in a liquid formulation. Since the buffer does not contain any stabilizing proteins or dyes, the capsids can be used in various applications, including dot blot, western blot, ELISA, qPCR, ddPCR and cell-based assays. Our material contains only a very low remaining amount of iodixanol allowing DLS-SLS-UV/Vis analysis. The lot-specific titers were assigned using AAVrh10 ELISA (PRAAV10) according to our internally established standard material* in combination with AAVrh74 kit control (PRAAV74-C) and PCR using eGFP primers. Our comprehensive quality control ensures well-characterized capsid material, which can be implemented as reference material in a variety of assays to prove the validity of the corresponding assay.
PROGEN provides eGFP-filled AAV standard material for the AAV serotypes 1, 2, 3, 5, 6, 8, 9, rh10, and rh74.
*Our internal standard material for each serotype was characterized according to the protocol described in our poster Developing Reliable AAV Standards for ELISA (available in the downloads tab). Data on the establishment of standard material for specific serotypes can be found as part of the performance data for the corresponding ELISAs or can be provided upon request.

Product images

AAVrh74 standard material (eGFP)



SDS PAGE with AAVrh74 eGFP-filled capsids. The AAVrh74 VP1, VP2 and VP3 proteins were separated on a 10% SDS PAGE and visualized by Pierce Silver stain kit. Only VP1, VP2 and VP3 proteins in the correct stoichiometry of 1:1:10 are detectable indicating a purity of the AAV preparation of >95%.


Transduction assay of AAVrh74 standard material (eGFP) using HeLa cells. HeLa cells (50 µl) were plated in 96-well plates at 200,000 cells/ml in DMEM + 10% FCS. 50 µl of eGFP-filled AAVrh74 capsids (1.7E+10 vg/ml) were added to the cells and incubated for 48 h at 37°C and 5% CO2. After incubation GFP-expression was visualized with a fluorescence microscope.