

CERTIFICATE OF ANALYSIS

Purified AAV5-CMV-GFP and AAV5-Empty (Lot# 21-240 and Lot#21-584)

Storage Conditions

The AAV vectors should be stored at -80°C for long term usage. When storing for frequent use, 4°C is recommended. It's not recommended to store AAV vectors at -20°C.

Shelf Life

5 years when stored at -80°C. Minimize the freeze and thaw cycle.

Shipping Conditions

Dry ice overnight

Description

AAV5-CMV-GFP was produced in insect Sf9 cells by dual infection with rBV-pFB inCap5inRep-kozak-updated-hr2 (Clone ID: V295) (Fig. 2) and rBV-V445-pFB-CMV-GFP (Fig. 3). For AAV5-Empty, only rBV-V295 was used. The vectors were purified through 2 rounds of CsCl ultracentrifugations. The CsCl band was removed through buffer exchange with 2 PD-10 desalting columns.

QPCR Titer

2E+13 vg/mL

The titer of AAV5-CMV-GFP was determined with QPCR method using primers/probe corresponding to the AAV2 ITR. The titer of AAV5-Empty is determined by nanodrop and SDS-PAGE analysis.

Quality Control Data

The AAV vector was formulated in 1xPBS buffer pH7.4, containing 0.001% pluronic F-68, and sterilized with 0.22µm low protein-binding filter. SDS-PAGE and SimplyBlue Staining (Invitrogen) verified the purity of the vectors (Fig. 1). QPCR analysis determines the titers of the AAV samples.



Μ	AAV8-CNTRL	AAV5-CMV-GFP
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Fig. 1. SDS-PAGE and Instant Blue Staining of purified AAV5-CMV-GFP (Lot#21-240). Lane M, protein ladder; lane 1, AAV8 control; lane 2, AAV5-CMV-GFP (#21-240)

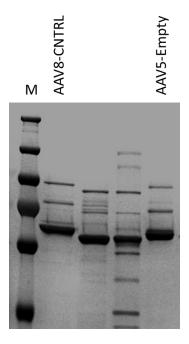


Fig. 2. SDS-PAGE and Instant Blue Staining of purified AAV5-Empty (Lot#21-584). Lane M, protein ladder; lane 1, AAV8 control; lane 4, AAV5-Empty

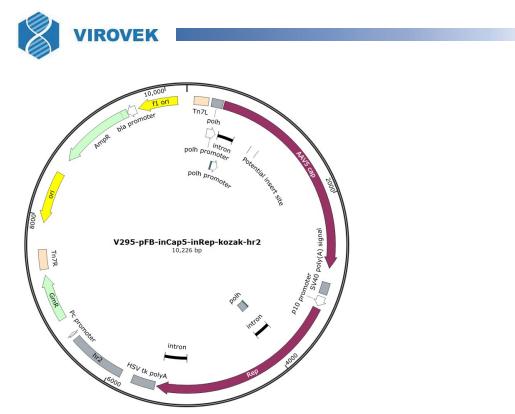


Fig. 3. Diagram of plasmid used to generate rBV-V295-pFB-inCap5-inRep-kozak-hr2

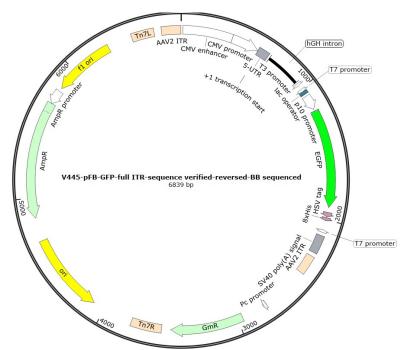


Fig. 4. Diagram of plasmid used to generate rBV-V445-pFB-GFP.

Approved by: <u>Santanu Raychaudhuri</u> Date: January 19, 2023