Product Data Sheet

pLenti-AAVR-Puro Vector

CATALOG NUMBER: AAV-450 **STORAGE:** -20ºC

QUANTITY AND CONCENTRATION: 10 µg at 0.25 µg/µL in TE

Background

Adeno-associated viruses (AAVs) are derived from defective parvoviruses, which depend on essential helper functions provided by other viruses, such as adenovirus and herpes virus, for efficient viral replication and propagation. AAV has no etiologic association with any known diseases and has been successfully used to establish efficient and long-term gene expression in vivo in a variety of tissues without significant cellular immune responses or toxicity. AAV has a single-stranded DNA genome which consists of approximately 4.7 kb. All characterized AAV serotypes share three key features, including two copies of AAV terminal repeats (ITRs), one *rep* region and one *cap* region.

Recently, through genome-wide screening for genes essential to AAV transduction, type I transmembrane protein, KIAA0319L (renamed as AAV receptor (AAVR)) was implicated as the key receptor for entry of a panel of AAV serotypes into representative cell types and, in vivo, in mice (Ref. 4). AAVR has a single transmembrane region and a short C-terminal cytoplasmic domain required for retrograde trafficking to the trans-Golgi network. Its ectodomain, which binds AAV's capsid, comprises five tandem Ig-like PKD (polycystic kidney disease) domains, a MANEC domain, and an N-terminal signal peptide (Figure 1).

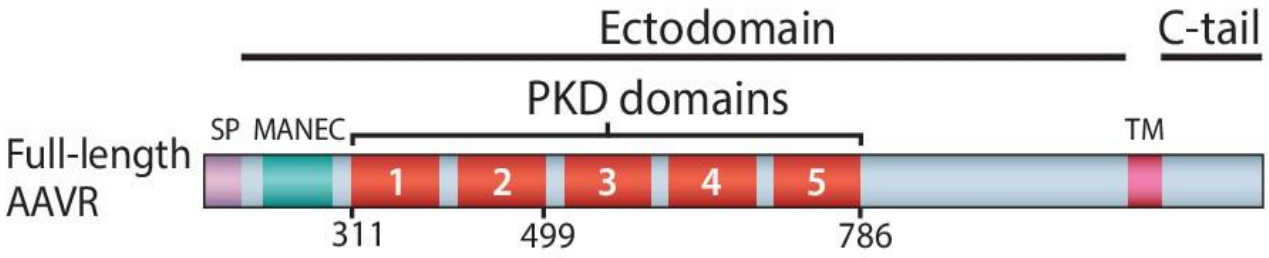

Figure 1. Schematic representation of the domain structure of AAVR

Related Products

1. AAV-100: 293AAV Cell Line
2. AAV-200: ViraDuctin™ AAV Transduction Kit
3. VPK-140: ViraBind™ AAV Purification Kit
4. VPK-145: QuickTiter™ AAV Quantitation Kit
5. VPK-402: AAV Helper Free Packaging System

pLenti-AAVR-Puro Vector

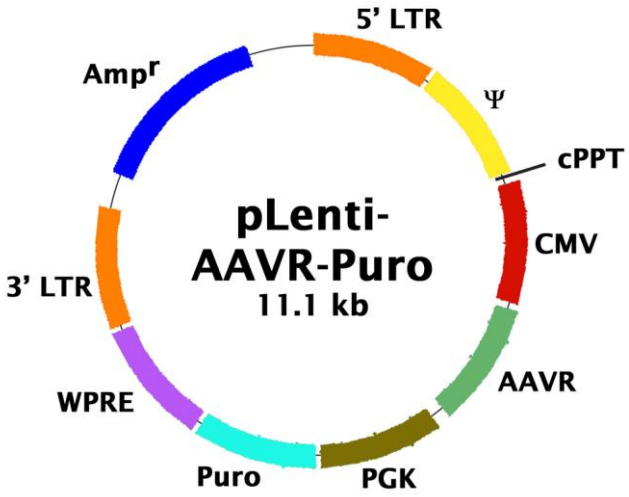

Figure 2. Schematic representation of pLenti-AAVR-Puro Vector (11092 bp, Ampicillin-resistant). EcoRI Digestion: 1147 bp + 2661 bp + 7284 bp.

Vector Features:

45-1909:	5' RSV/LTR, RRE, cPPT
1980-2537:	CMV
2598-5771:	AAVR-DYKDDDDK
5774-6366:	WPRE
6920-7519:	PuroR gene
7655-7891:	3' LTR
9051-9911:	AmpR gene

Lentivirus Production

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (cat.# LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (pLenti-AAVR-Puro: pCMV-VSV-G:pRSV-REV:pCgpV).

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4ºC over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 µm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80ºC in aliquots.

Post-Packaging Considerations

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

1. **Concentration and purification of your lentivirus**: Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection.
2. **Measure the titer of your lentivirus**: This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses

exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

References

1. Auricchio, A., Hildinger, M., O'Connor, E., Gao, G. P. and Wilson, J. M. (2001) *Hum Gene Ther* **12**:71–6.
2. Brument, N., Morenweiser, R., Blouin, V., Toublanc, E., Raimbaud, I. et al. (2002) *Mol Ther* **6**:678–86.
3. Clark, K., Liu, X., McGrath, J., and Johnson, P. (1999) *Hum. Gene Ther*., **10**, 1031-1039.
4. Pillay S., Meyer N.L., Puschnik A.S., Davulcu O., Diep J., Ishikawa Y., Jae L.T., Wosen J.E., Nagamine C.M., Chapman M.S., et al. (2016) *Nature.* **530**, 108–112.
5. Dudek A.M., Pillay S., Puschnik A.S., Nagamine C.M., Cheng F., Qiu J., Carette J.E., Vandenberghe L.H. (2018) *J. Virol.* **92**, 2213-17.
6. Zhang R., Cao L., Cui M., Sun Z., Hu M., Zhang R., Stuart W., Zhao X., Yang Z., Li X., et al. (2019) *Nat. Microbiol.* **4**, 675-682.
7. Pillay S., Zou W., Cheng F., Puschnik A.S., Meyer N.L., Ganaie S.S., Deng X., Wosen J.E., Davulcu O., Yan Z., et al. (2017) *J. Virol.* **91**, 391-17.

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