

scAAV2-GFP Control Virus

CATALOG NUMBER: AAV-332 **STORAGE:** -80°C; avoid repeat freeze/thaw cycles

QUANTITY AND CONCENTRATION: 50 µL, 1×10^{12} GC/mL in PBS/0.001% Pluronic F-68

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. In the AAV Helper-Free System, most of the adenovirus gene products required for the production of infective AAV particles are supplied on the plasmid pHelper (i.e. E2A, E4, and VA RNA genes) that is co-transfected into cells with human AAV vector DNA. The remaining adenoviral gene product is supplied by the 293 host cells, which stably express the adenovirus E1 gene. The *rep* and *cap* genes have been removed from the viral vector that contains AAV-2 ITRs and are supplied *in trans* on the plasmid pAAV-RC. Variations on capsid protein confer serotypes of AAV to have different tropism. The expression of the luciferase reporter in the provided AAV is driven by a CMV promoter.

The AAV transduction process includes viral binding and entry, intracellular trafficking, nuclear transport, and viral second strand DNA synthesis. The viral second strand DNA synthesis has been shown to be the rate limiting step, which leads to inefficient transduction by AAV vectors. In scAAV (self-complementary AAV), the right ITR contains a deletion of D-sequence (the packaging signal) and a terminal resolution site mutation (Δ trs), which prevent Rep-mediated nicking and force packaging of dimer or self-complementary genomes. To make dsAAV from an scAAV vector renders much improved transduction for both *in vitro* and *in vivo* experiments (Wang *et al*, Ref 11).

In Vitro Infection Methods

When designing AAV experiments, we recommend using a reporter virus such as AAV-GFP to determine optimal serotype for infection of your cell type. (AAV infection is cell type dependent; the required MOI for different cell types needs to be determined.)

1. Seed target cells in a 24-well or 12-well plate one day before infection.
2. Infect cells with recombinant AAV at desired MOI.

Note: For cells that are readily infectible, we recommend starting at 5,000 to 10,000 GC/cell.

3. (Optional) Coinfect cells with wild type Ad5 at MOI of 1.
4. After 48 to 72 hrs, perform appropriate assays to determine positive cells.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Precautions must be taken to avoid direct contact with viral supernatants.

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. Graham, F. L., Smiley, J., Russell, W. C. and Nairn, R. (1977) *J Gen Virol* **36**:59-74.
5. Grimm, D. and Kleinschmidt, J. A. (1999) *Hum Gene Ther* **10**:2445-50.
6. Matsushita, T., Elliger, S., Elliger, C., Podsakoff, G., Villarreal, L. et al. (1998) *Gene Ther* **5**:938-45.
7. McCarty, D. M., Monahan, P. E. and Sumulski, R. J. (2001) *Gene Therapy* **8**:1248-1254.
8. Rabinowitz, J. and Samulski, R. J. (1998) *Curr. Opin. Biotechnol.*, **9**, 470-475.
9. Russell, D. W., Alexander, I. E. and Miller, A. D. (1995) *Proc Natl Acad Sci U S A* **92**:5719-23.
10. Summerford, C., and Samulski, R. J. (1999) *Nat. Med.*, **5**, 587-588.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2017-2018: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.