scAAV1-GFP Control Virus

CATALOG NUMBER: AAV-331 **STORAGE:** -80°C; avoid repeat freeze/thaw cycles

QUANTITY AND CONCENTRATION: 50 µL, 1 x 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 (Δtrs), 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

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