PRODUCT INFORMATION

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Product name	Product #	Lot#	Viral Genome	Volume	Expiration date
rAAV2_mOaPAC 360	BD1180	20200401	2.0E+11 VG	20 µL	31MAR2022

CERTIFICATE OF ANALYSIS

Manufacture Date: March 2020

Test	Specification	Results
Plasmid Characterization	Accuracy	Correct
Viral Genome (VG) Copy Titer	>1.0E+13 VG/mL	1.05E+13 VG/mL
Endotoxin	<0.05 Unit/mL	0.000 Unit/mL

Plasmid Characterization: there are three plasmids: AAV cis plasmids, containing the vector genome, AAV trans plasmids containing rep and cap genes, and adenovirus helper plasmids encoding essential adenovirus functions, which are used for the AAV production. Full plasmid characterization includes determination of concentration, determination of A260/280 ratio, detection of endotoxin, and plasmid identity. In the plasmid identity assay, the presence of each functional DNA element is determined by sequencing and the correct restriction pattern is verified with the plasmid map and the seed DNA preparation. For AAV cis-plasmids, these elements include the 5' and 3' ITR, transgene, promoter, polyadenylation sequence. For AAV trans plasmids, the presence of the correct cap gene is verified by full length sequencing.

Viral Genome (VG) Copy Number Titration: Real time PCR via Applied Biosystems 7900HT are employed to determine the viral genome (VG) copy number of AAV vector lots as a measure of AAV particles with full genome content. A number of standards, validation samples (both viral and plasmid) and controls (for background and DNA contamination) have been used in this assay.

Endotoxin Assay: The endotoxin assay is carried out using the Limulus Amebocyte Lysate (LAL) gel-clot method. Contaminating endotoxin in plasmid preparations can affect transfection efficiencies as well as alter the immunogenic properties of the final product.

All the pre-packaged recombinant adeno associated viruses provided by Hamamatsu Photonics K.K. are for research application purposes ONLY!

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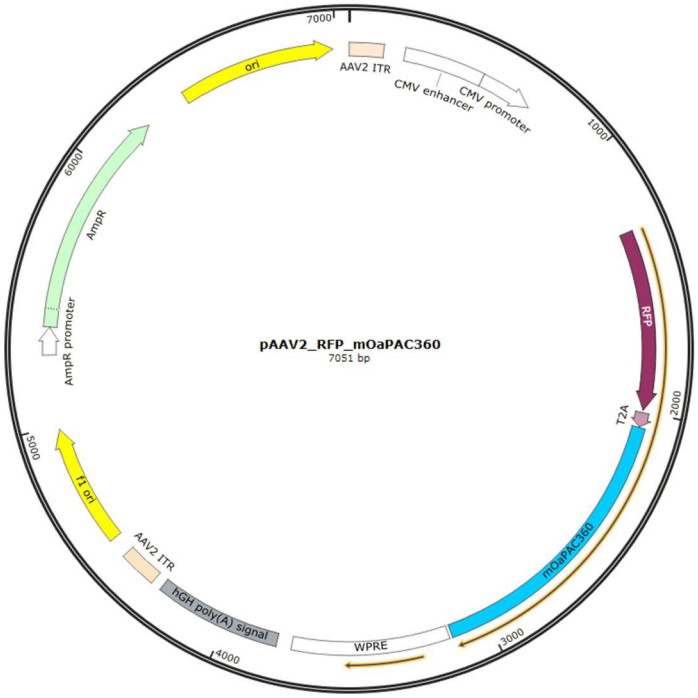
The genetic map of AAV serotype 2 cisplasmid “pAAV2_RFP_mOaPAC360”



Location of features

AAV2 ITR: 1-130

CMV enhancer: 210-513

CMV promoter: 514-716

kozak: 1314-1319

RFP: 1320-1994

T2A: 2004-2057

mOaPAC360: 2061-3140

WPRE: 3152-3740

hGH poly(A) signal: 3798-4274

AAV2 ITR: 4314-4454

F1 ori: 4529-4984

AmpR promoter: 5266-5370

AmpR: 5371-6231

ori: 6402-6990

Contents and Storage:

AAV stocks are supplied in liquid form at indicated titer. The storage solution PBS / 5% glycerol.
Store at -80℃.
If desired, aliquot viral stock upon arrival, and store those aliquots at -80℃ freezer immediately.
DO NOT FREEZE AND THAW REPEATEDLY.

Red fluorescent protein (RFP) is a marker for PAC gene expressing cells.
The excitation maximum is 555 nm, and the emission maximum is 584 nm.

References

Hirano *et al.* (2019) “The C-terminal region affects the activity of photoactivated adenylyl cyclase from *Oscillatoria acuminata*.” *Scientific Reports* 9, Article number: 20262.

Ohki *et al.* (2016) “Structural insight into photoactivation of an adenylate cyclase from a photosynthetic cyanobacterium.” *PNAS* 113 (24) 6659-6664.

Infection Protocol of recombinant Adeno-associated Virus (rAAV)

In-vitro Infection Protocol:

1. Prepare virus-containing media:
Thaw viral stock at either room temperature or on ice.
Add desired amount of virus to growth media to achieve the desired multiplicity of infection (MOI).
AAV Genome particles (VG) to be used = MOI x number of cells to be infected
e.g. If you intend to infect 1 million cells using MOI of 10,000, you need [1.0E+4] x [1.0E+6] = 1.0E+10 VG for the infection. If the original stock is 1.0E+13 VG /ml, then you will need 1.0 ul of the original stock for the dilution.

2. Infecting cells with AAV:
Remove the original cell culture media, and add the above AAV-containing media to cell culture. Below is a general guideline for the amount of media used:

24-well plate: 0.2-0.3 ml
12-well plate: 0.5-0.8 ml
6-well plate: 1-1.5 ml/well
60mm-plate: 3-4 ml/plate
10cm-plate: 8-12 ml/plate

Incubate cells with the virus-containing media for 6-12 hours, or as long as you wish.
(Optional), you could remove virus-containing media and replace it with fresh, desired media.
The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. The goal is to get 100% of infection without causing any undesired effects. The optimal concentration differs dramatically between cell types for different serotype of AAV. A range of 2,000-10,000 MOI is used for most cell lines, but up to 500,000 MOI may be used for some cells.
To determine this optimal concentration of virus for your study, you could conduct pilot testing in your cell line by using reporter AAV like AAV-GFP.

3. Co-infection with Adenovirus
Together with diluted AAV, co-infect with wild-type adenovirus type 5 at MOI of 1~100. Co-infection with wild type adenovirus will significantly (5-100 folds) boost transduction efficiency and gene expression level.

Note:
(1) AAV stock can be added directly to cells in culture medium (in the presence or absence of serum).
(2) It is not necessary to remove viruses, change or add medium following infection, although viruses can be removed after 6-12 hours post infections.
(3) It can take 3-7 days after the AAV infection to detect the gene over-expression
(4) Co-infection with wild type adenovirus will significantly boost AAV's transduction efficiency and transgene expression level.

In-vivo Infection Protocol:

The following protocol is given for stereotaxic gene delivery of rAAV to the rodent brain.

Materials:

- Super purified rAAV in PBS, in vivo grade.
- Ethanol 70%.
- Mice or rats.
- Anesthetics and analgesics (e.g., ketamine etc).
- Sterile PBS
- Bone wax
- Triple antibiotic ointment

Procedures:

1. Anesthetize and fix animal in stereotaxic apparatus.
2. Make incision and locate bregma.
3. Surgery - Preparation of craniotomy by referring to the standard stereotaxic coordinates of mouse or rats. The craniotomy is drilled using a hand-held drill. The following table is an example of stereotaxic coordinates.
4. Injection of rAAV for a single injection. Place the injection micropipette into the holder of the stereotaxic arm. Fill the micropipette with 3 µL rAAV. Then slowly lower the micropipette to the desired z coordinate of the injection site. Apply pressure steadily via a pump or syringe to inject 100~500 nL rAAV at >1E+13 VG/mL within 60~90 seconds.
5. After recovery of animal, feed the animal as usual until the transgene expression is checked 4 weeks after rAAV injection.

Targeted region adult mouse brain	Rostral (+) caudal (–) (mm)	Lateral (mm)	Ventral (mm)
Subthalamic nucleus	- 1.9	1.6	4.4
Dorsal hippocampus, CA1	- 2.1	2.0	1.4
Basolateral amygdala	- 1.5	2.75	4.75
Lateral ventricle	+ 0.5	0.75	2.5
Nucleus accumbens, core	+ 1.1	1.2	4.5

Light condition for PAC activation:

PAC has the blue light using flavin (BLUF) domain for the light-triggered switch and therefore all the type of blue light module (the maximum emission wavelength: around 450 nm) is recommended.
For more information, see the references below:

1) Hirano *et al.* (2019) “The C-terminal region affects the activity of photoactivated adenylyl cyclase from *Oscillatoria acuminata*.” *Scientific Reports* 9, Article number: 20262.

2) Ohki *et al.* (2016) “Structural insight into photoactivation of an adenylate cyclase from a photosynthetic cyanobacterium.” *PNAS* 113 (24) 6659-6664.

3) Zhou *et al.* (2016) “Photoactivated adenylyl cyclase (PAC) reveals novel mechanisms underlying cAMP-dependent axonal morphogenesis.” *Scientific Reports* 5, Article number: 19679.