

AAVpro® CRISPR/Cas9 Vector Set

Catalog No.	Amount	Lot Number
632610 (Not sold separately) Sold as a part of 632608, 632609	1 Set	Specified on product label.

Description

When used as part of the AAVpro CRISPR/Cas9 Helper Free System (AAV2), the AAVpro CRISPR/Cas9 Vector Set allows for adeno-associated virus (AAV) delivery of the genes encoding the components necessary for CRISPR/Cas9-mediated genome editing in mammalian cells. The vector set consists of two plasmids (pAAV-Guide-it-Up and pAAV-Guide-it-Down) that each contain a truncated, upstream or downstream portion of the Cas9 gene with a 1.6-kb region of homology. Due to the nature of the AAV genome, when target cells are transduced with both viruses (Up and Down), recombination occurs at the region of homology to create a full-length Cas9 gene with an upstream CMV promoter. pAAV-Guide-it-Down is supplied pre-linearized for simple cloning of a target-specific sgRNA sequence under the control of the human U6 promoter.

Package Contents

- 20 µl pAAV-Guide-it-Up Vector (500 ng/µl)
- 20 µl pAAV-Guide-it-Down Vector (Linear) (7.5 ng/µl)

Storage Conditions

- Store plasmids at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze-thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at takarabio.com/manuals
The following documents apply to this product:

- AAVpro CRISPR/Cas9 Helper Free System (AAV2) User Manual
- AAVpro CRISPR/Cas9 Vector Information

Takara Bio USA, Inc.

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Certificate of Analysis

Cat. No. 632610

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Propagation in *E. coli*

- Recommended host strain: Stellar™ Competent Cells (Cat. No. 636763)
- Selectable marker: Plasmids confer resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Quality Control Data

Cloning efficiency

2µl of pAAVGuide-it-Down (Linear) was ligated with Guide-it Control Annealed Oligos, following the protocol outlined in the User Manual. After ligation, 10 µl of the reaction was used to transform 100 µl of Stellar Competent Cells, and serial dilutions were plated on LB agar + Ampicillin plates. The total number of colonies in the positive reaction (with the annealed control oligos) was calculated to be 10,000 or more colonies, and the negative reaction (without the annealed control oligos) yielded ≤3% of the positive reaction. Sequence analysis using the Guide-it Sequencing Primer 1 verified that 80% or more of the colonies from the positive reaction had a correct insert.

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzyme(s)	Size (kb)
pAAV-Guide-it-Up	EagI	7.1 kb
	HindIII	4.5 kb, 2.6 kb
pAAV-Guide-it-Down	EcoRV	6.3 kb
	EagI	5.2 kb, 1.2 kb

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280} : 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

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CATALOG NO.

632610

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STATEMENT M83

This product is the subject of the claims of US patent No. 9422576 and its foreign counterparts.

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