

pAAV-Guide-it-1 Vector (Linear)

Catalog No.	Amount	Lot Number
632621 (Not sold separately) Sold as a part of 632618 & 632619	20 µl	Specified on product label.

Description

When used as part of the AAVpro® CRISPR/SaCas9 Helper Free System (AAV2), the pAAV-Guide-it-1 Vector 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 contains the Cas9 gene from *Staphylococcus aureus* (SaCas9) expressed from an upstream CMV promoter. SaCas9 has a genome editing efficacy similar to the more commonly used *Streptococcus pyogenes* Cas9 (SpCas9) and is about 1 kb shorter, which allows it to be packaged into a single AAV vector. pAAV-Guide-it-1 is supplied pre-linearized for simple cloning of a target-specific sgRNA sequence under control of the human U6 promoter.

Package Contents

- 20 µl pAAV-Guide-it-1 Vector (Linear) (7.5 ng/µl)

Storage Conditions

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

Expiration Date

- Specified on product label.

Storage Buffer

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

Shipping Conditions

- Dry ice

Product Documents

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

- AAVpro CRISPR/SaCas9 Systems User Manual
- pAAV-Guide-it-1 (Linear) Vector Sequence in GenBank Format

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

Certificate of Analysis

Cat. No. 632621

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Quality Control Data

Cloning Efficiency

2 μ l of pAAVGuide-it-1 Vector (Linear) was ligated with Guide-it™ Control Annealed Oligos v3 following the protocol outlined in the Guide-it CRISPR/SaCas9 Systems User Manual. After ligation, 10 μ l of the reaction was used to transform 100 μ l 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, while the negative reaction (without the annealed control oligos) yielded $\leq 5\%$ of the positive reaction. Sequence analysis using the Guide-it Sequencing Primer 1 verified that at least 80% of the colonies from the positive reaction had a correct insert.

Plasmid Identity & Purity

- Prior to linearization, digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzymes	Fragments
NotI	7,046 bp
BamHI	4,011 bp & 3,035 bp

- 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.

632621

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