

pGuide-it-sgRNA1 Vector (Linear)

Catalog No.	Amount	Lot Number
632614 (Not sold separately) Sold as a part of 632612, 632613	20 μ l	Specified on product label.

Description

The pGuide-it-sgRNA1 Vector (Linear) is provided as part of the Guide-it™ CRISPR/Cas9 Vector System. This kit is designed for cloning and expression of a target single guide RNA (sgRNA) for mammalian gene modification studies using CRISPR/Cas9 technology. This plasmid is supplied pre-linearized for simple insertion of the gene-targeting encoding section of the sgRNA downstream of a human U6 promoter. There is no fluorescence reporter or mammalian selection marker on this vector. It is used for the transient transfection of a packaging cell line in order to produce vesicles containing a protein complex of Cas9 and an sgRNA of interest.

Package Contents

- 20 μ l pGuide-it-sgRNA1 Vector (Linear) (7.5 ng/ μ l)

Storage Conditions

- Store 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:

- Guide-it CRISPR/Cas9 Vesicle Production System User Manual
- pGuide-it-sgRNA1 Vector (Linear) Vector Information

Propagation in *E. coli*

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

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Quality Control Data

Cloning Efficiency

The pGuide-it-sgRNA1 Vector (Linear) (2 µl) was ligated with the Guide-it Control Annealed Oligos v2 following the protocol outlined in the Guide-it CRISPR/Cas9 Gesicle Production System User Manual. After ligation, 5 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 resulting from the positive reaction (with Annealed Oligos) was calculated to be >5,000, whereas the negative reaction (without Annealed Oligos) yielded <6.5% of the positive reaction. Sequence analysis using the Guide-it Sequencing Primer 1 verified that >80% of the colonies from the positive reaction had the correct insert.

Plasmid Identity & Purity

- Prior to linearization, digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 1% agarose/EtBr gel:

Vector	Enzyme(s)	Size (kb)
pGuide-it-sgRNA1	HindIII	3,042 bp
	HaeII	1,871 bp, 801 bp, 370 bp

- Vector identity was confirmed by sequencing.
- A₂₆₀/A₂₈₀: 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.

632614

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