# Certificate of Analysis



# pGuide-it-ZsGreen1 Vector (Linear)

Catalog No. Amount Lot Number

632603 (Not sold separately) 20 µl Specified on product label. Sold as a part of 632601

## **Description**

The pGuide-it-ZsGreen1 Vector (Linear) is provided as part of the Guide-it<sup>TM</sup> CRISPR/Cas9 System (Green). This kit is designed for cloning and expression of a target single guide RNA (sgRNA) for mammalian gene modification studies using CRISPR/Cas9 technology. Cas9 nuclease and ZsGreen1 fluorescent protein are co-expressed from a bidirectional CMV promoter. The plasmid is supplied pre-linearized for simple insertion of the genome-targeting encoding section of the sgRNA downstream of a human U6 promoter.

## **Package Contents**

• 20 μl pGuide-it-ZsGreen1 Vector (Linear) (7.5 ng/μl)

## **Storage Conditions**

- Store at  $-20^{\circ}$ C.
- 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  $(-70^{\circ}C)$ 

#### **Product Documents**

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

- Guide-it CRISPR/Cas9 System User Manual
- pGuide-it-ZsGreen1 Vector (Linear) Vector Information
- pGuide-it-ZsGreen1 (Linear) Vector Sequence in GenBank Format

### Propagation in *E. coli*

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

Sold as a part of 632601

## **Quality Control Data**

## **Cloning efficiency**

 $2 \mu l$  of pGuide-it-ZsGreen1 (Linear) was ligated with Guide-it Control Annealed Oligos, following the protocol outlined in the Guide-it CRISPR/Cas9 System User Manual. After ligation,  $10 \mu l$  of the reaction was used to transform  $100 \mu l$  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, and the negative reaction (without the annealed control oligos) yielded  $\leq 3\%$  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**

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

Vector	Enzymes	Fragments
pGuide-it-ZsGreen1	EcoRI	8.3 kb
	NdeI	5.1 kb, 3.2 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.

(110723) Page 2 of 2



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CATALOG NO.

632603

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### STATEMENT 391

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