Certificate of Analysis



pGuide-it-tdTomato Vector (Linear)

Catalog No. Amount Lot Number

632604 (Not sold separately) 20 μl Specified on product label.

Sold as a part of 632601

Description

The pGuide-it-tdTomato Vector (Linear) is provided as part of the Guide-itTM CRISPR/Cas9 System (Red). 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 tdTomato 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-tdTomato Vector (Linear) (7.5 ng/μl)

Storage Conditions

- Store at -20° 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

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-tdTomato (Linear) Vector Sequence in GenBank Format

Propagation in E. coli

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

pGuide-it-tdTomato Vector (Linear) (Not sold separately)

Sold as a part of 632602

Quality Control Data

Cloning efficiency

2 μ l of pGuide-it-tdTomato (Linear) was ligated with Guide-it Control Annealed Oligos (part of the Guide-it Ligation Components, Cat. No. 632605), following the protocol outlined in the Guide-it CRISPR/Cas9 System 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, 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 (Guide-it Ligation Components, Cat. No. 632605) 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-tdTomato	EcoRI	9.1 kb
	NdeI	5.1 & 4.0 kb

Vector identity was confirmed by sequencing.

 \bullet 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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pGuide-it-tdTomato Vector (Linear)

CATALOG NO.

632604

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Canada Patent Nos. 2,891,347 and 2,977,152; China Patent No. CN105142669; European Patent Nos. EP 2 928 496 B1; EP 3 138 910 B1, 3 138 911 B1, EP 3 138 912 B1, EP 3 360 964 B1, EP 3 363 902 B1; Israel Patent No. IL238856; Singapore Patent No. 11201503824S; South Korea Patent Nos. 10-1844123 and 10-2006880; and U.S. Patent Application Serial Nos. 15/188,911; 15/188,924; 15/188,927; 15/188,931; and 15/456,204 (the "Patent Rights"). The purchase of this Product conveys to you (the "Buyer") the NON-TRANSFERABLE right to use the Product for Licensed Research Use (see definition below) subject to the conditions set out in this License Agreement. 1. "Licensed Research Use" means any use for research purposes, except: (i) Buyer may not sell or otherwise transfer the Product (including without limitation any material that contains the Product in whole or part) or any Related Material to any other third party (except that you may transfer the Product, or any Related Material to a bona fide collaborator or contract research organization), or use the Products or any Related Material to perform services for the benefit of any other third party; (ii) Buyer may use only the purchased amount of the Product and components of the Product, and shall use any Related Material, only for your internal research within the Field, and not for any Commercial Purposes; (iii) Buyer shall use the Product and any Related Material in compliance with all applicable laws and regulations, including without limitation applicable human health and animal welfare laws and regulations; and (iv) the Buyer shall indemnify, defend, and hold harmless SIGMA and their current and former directors, officers, employees and agents, and their respective successors, heirs and assigns (the "Indemnities") against any liability, damage, loss, or expense (including without limitation reasonable attorneys' fees and expenses) incurred by or imposed upon any of the Indemnitees in connection with any claims, suits, investigations, actions, demands or judgments arising out of or related to the exercise of any rights granted to the Buyer hereunder or any breach of this License Agreement by such Buyer. 2. For purposes of Section 1 above, the following definitions shall apply: "Commercial Purposes" means (a) the practice, performance or provision of any method, process or service, or (b) the manufacture, sale, use, distribution, disposition or importing of any product, in each case (a) or (b) for consideration, or on any other commercial basis. "Field" means use as a research tool for research purposes; provided, however, that notwithstanding the foregoing, the Field shall expressly exclude (a) any in vivo and ex vivo human or clinical use, including, without limitation, any administration into humans or any diagnostic or prognostic use, (b) the creation of transgenic rodent models and/or derivatives thereof (including, but not limited to, rodents' cells and rodents' organs) by for-profit entities, (c) any in vivo veterinary or livestock use, or non-research agricultural use, or (d) use as a testing service, therapeutic or diagnostic for humans or animals. "Related Materials" means any progeny, modification or derivative of a Product. 3. Your right to use the Product will terminate immediately if you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, Related Materials, and components thereof in your control, and notify SIGMA of such in writing. For information on purchasing a license to this Product for purposes other than Licensed Research Use, contact your local SIGMA sales representative, or call +1 800-325-3010.

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Takara Bio USA, Inc.

2560 Orchard Parkway, San Jose, CA 95131, USA U.S. Technical Support: technical support@takarabio.com

United States/Canada **Asia Pacific** Europe Japan +1.650.919.7300

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