

pGuide-it-ZsGreen1 Vector (Linear)

Catalog No.	Amount	Lot Number
632603 (Not sold separately) Sold as a part of 632601	20 μ l	Specified on product label.

Description

The pGuide-it-ZsGreen1 Vector (Linear) is provided as part of the Guide-it™ 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 μ l pGuide-it-ZsGreen1 Vector (Linear) (7.5 ng/ μ l)

Storage Conditions

- Store at -20°C
- 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 System User Manual
- pGuide-it-ZsGreen1 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

Certificate of Analysis

Cat. No. 632603

pGuide-it-ZsGreen1 Vector (Linear) (Not sold separately)

Sold as a part of 632601

Quality Control Data

Cloning efficiency

2 µl 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 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

- 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-ZsGreen1	EcoRI	8.3 kb
pGuide-it-ZsGreen1	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.

pGuide-it-ZsGreen1 Vector (Linear)

CATALOG NO.

632603

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product's web page at <http://www.takarabio.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

STATEMENT 272

This product ("Product") and its use, is the subject of U.S. Patents 8,697,359 and 8,771,945 and pending U.S. Patent applications. The purchase of the Product conveys to the buyer the non-transferable right to use Product(s) purchased from Takara Bio USA, Inc. or its Affiliates, and any progeny, modification or derivative of a Product, or any cell or animal made or modified through use of a Product, or any progeny, modification or derivative of such cell or animal ("Related Material"), solely for research conducted by the buyer in accordance with all of the following requirements. No right is given to use this Product or Related Material for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. The buyer shall not sell or otherwise transfer Products (including without limitation any material that contains a Licensed Product in whole or part) or any Related Material to any other person or entity, or use Products or any Related Material to perform services for the benefit of any other person or entity, (ii) the buyer shall use only the purchased amount of the Products and components of the Products, and shall use any Related Material, only for its internal research and not for (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 ("Commercial Purpose"), (iii) the buyer shall use Licensed Products 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 MIT, Harvard and The Broad and their current and former trustees, directors, officers, faculty, affiliated investigators, students, employees, and agents and their respective successors, heirs and assigns ("Indemnitees"), 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, or any breach of the rights granted hereunder by the buyer.

STATEMENT 391

LIMITED USE LABEL LICENSE: RESEARCH USE ONLY Notice to Purchaser: This product is subject to a

Takara Bio USA, Inc.

1290 Terra Bella Avenue, Mountain View, CA 94043, USA

U.S. Technical Support: techUS@takarabio.com

United States/Canada

Asia Pacific

Europe

Japan

800.662.2566

+1.650.919.7300

+33.(0)1.3904.6880

+81.(0)77.565.6999

11/17/2020

license granted to Takara Bio USA, Inc. and its Affiliates from Caribou Biosciences, Inc., and this product is transferred to the end-user purchaser (“Purchaser”) subject to a “Limited Use Label License” conveying to the Purchaser a limited, non-transferable right to use the product, solely as provided to Purchaser, together with (i) progeny or derivatives of the product generated by the Purchaser (including but not limited to cells), and (ii) biological material extracted or derived from the product or its corresponding progeny or derivatives (including but not limited to cells) (collectively, the product, and (i) and (ii) are referred to as “Material”) only to perform internal research for the sole benefit of the Purchaser. The Purchaser cannot sell or otherwise transfer Material to a third party or otherwise use the Material for any Excluded Use. “Excluded Use” means any and all: (a) commercial activity including, but not limited to, any use in manufacturing (including but not limited to cell line development for purposes of bioproduction), product testing, or quality control; (b) preclinical or clinical testing or other activity directed toward the submission of data to the U.S. Food and Drug Administration, or any other regulatory agency in any country or jurisdiction where the active agent in such studies comprises the Material; (c) use to provide a service, information, or data to a third party; (d) use for human or animal therapeutic, diagnostic, or prophylactic purposes or as a product for therapeutics, diagnostics, or prophylaxis; (e) activity in an agricultural field trial or any activity directed toward the submission of data to the U.S. Department of Agriculture or any other agriculture regulatory agency; (f) high throughput screening drug discovery purposes (i.e., the screening of more than 10,000 experiments per day) as well as scale-up production activities for commercialization; (g) modification of human germline, including editing of human embryo genomes (with the sole exception of editing human embryonic stem (ES) cell lines for research purposes) or reproductive cells; (h) self-editing; and/or (i) stimulation of biased inheritance of a particular gene or trait or set of genes or traits (“gene drive”). It is the Purchaser’s responsibility to use the Material in accordance with all applicable laws and regulations. For information on obtaining additional rights, including commercial rights, please contact licensing@cariboubio.com or Caribou Biosciences, Inc., 2929 7th Street, Suite 105, Berkeley, CA 94710 USA, Attn: Licensing.

STATEMENT 396

Sigma-Aldrich CRISPR Use License Agreement This Product and its use are the subject of one or more of the following issued patents and patent applications: Australia Patent Nos. 2013355214; 2017204031; and 2018229489; 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

Takara Bio USA, Inc.

1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

11/17/2020

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.

TRADEMARKS:

©2020 Takara Bio Inc. All Rights Reserved.

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions.

Takara Bio USA, Inc.

1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada

Asia Pacific

Europe

Japan

800.662.2566

+1.650.919.7300

+33.(0)1.3904.6880

+81.(0)77.565.6999

11/17/2020