AccuTool™ pRGEN-Cas9

[Cat. No.] Please refer to the **Ordering Information**

Introduction

DNA-directed RNA-Guided Endonucleases (dRGENs) are efficient, affordable, and convenient tools for genome engineering experiments. The Cas9 expression plasmids can be used with custom sgRNA expression plasmids, and the Cas9 (WT or Nickase or Sniper) expression plasmid uses the CMV/T7 or EF1α promoter for strong transient expression of Cas9. The Cas9 Nickase (D10A) generates a DNA nick rather than a double-strand breaks (DSBs) and Sniper Cas9 improves specificity and on-target activity by reducing off-targeting. Plasmids can be delivered to your cell of interest by any standard methods like lipofection, nanoparticle, or electroporation to achieve highly efficient delivery. In addition, various pRGEN-Cas9 (WT or Nickase or Sniper) plasmids are provided according to the selection marker, such as Puro-RFP or Hygro-EGFP.

Applications

- Genome editing
- Drug discovery: CRISPR library screening, target validation
- Bioprocessing: Cell line engineering
- Agriculture: Plant breeding

Components

Components	Amount
Lyophilized pRGEN-Cas9 (WT or Nickase or Sniper)-CMV/T7 or Ef1α (none or Puro-RFP or Hygro-EGFP) Plasmid	5 μg or 50 μg

*** Note**: For research use only. Not for use in diagnostic or therapeutic procedures.

Specifications

Expression vectors for Cas9 gene (pRGEN-Cas9-CMV/T7 or Ef1α) are ampicillin-resistance and stable in general *E. coli* strains such as DH5α or XL1.

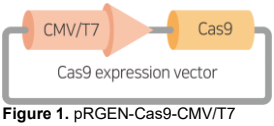


Figure 1. pRGEN-Cas9-CMV/T7

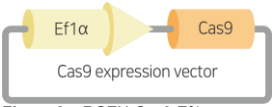
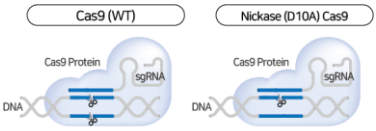


Figure 2. pRGEN-Cas9-Ef1α



	Cleavage	Nick	
		Single	Double
NHEJ (Insertion/deletion)	○	X	○ (insertion specificity)
HDR (Knock-in)	○	○ (reduced efficiency)	○

Storage

- *AccuTool*™ pRGEN-Cas9 is lyophilized and delivered at ambient temperature.
- Store at -20°C after adding distilled water (D.W.) or TE buffer. Do not store in a frost-free freezer.

Online Resources

Korean

English

Visit our **product page** for additional information and protocols.

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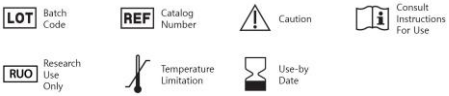
Ordering Information

Description				Cat. No.
AccuTool™ pRGEN-Cas9	CMV/T7		5 μg	ATS-0060
			50 μg	ATS-0061
		Puro-RFP	5 μg	ATS-0062
			50 μg	ATS-0063
		Hygro-EGFP	5 μg	ATS-0064
			50 μg	ATS-0065
	Ef1α		5 μg	ATS-0066
			50 μg	ATS-0067
		Puro-RFP	5 μg	ATS-0068
			50 μg	ATS-0069
		Hygro-EGFP	5 μg	ATS-0070
			50 μg	ATS-0071
AccuTool™ pRGEN-Cas9 nickase (D10A)	CMV/T7		5 μg	ATS-0072
			50 μg	ATS-0073
		Puro-RFP	5 μg	ATS-0074
			50 μg	ATS-0075
		Hygro-EGFP	5 μg	ATS-0076
			50 μg	ATS-0077
	Ef1α		5 μg	ATS-0078
			50 μg	ATS-0079
		Puro-RFP	5 μg	ATS-0080
			50 μg	ATS-0081
		Hygro-EGFP	5 μg	ATS-0082
			50 μg	ATS-0083
AccuTool™ pRGEN-sniper Cas9	CMV/T7		5 μg	ATS-0084
			50 μg	ATS-0085
		Puro-RFP	5 μg	ATS-0086
			50 μg	ATS-0087
		Hygro-EGFP	5 μg	ATS-0088
			50 μg	ATS-0089
	Ef1α		5 μg	ATS-0090
			50 μg	ATS-0091
		Puro-RFP	5 μg	ATS-0092
			50 μg	ATS-0093
		Hygro-EGFP	5 μg	ATS-0094
			50 μg	ATS-0095

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols



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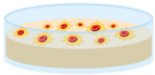
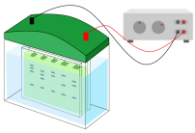
Experimental Procedures

Steps		Procedure Details	
Gene knock-out cell establishment			
1	 **Cell transfection**	1. Transfer CRISPR plasmids (sgRNA and Cas9) to target cells. • Any DNA delivery method (lipofection, electroporation, nucleofection, or microinjection) optimal for your cell line can be used to deliver CRISPR plasmids. • The recommended ratio of sgRNA expression plasmids and Cas9 gene expression plasmids is 1:1-5:1.	
2	 **(Optional) Detection of GFP or RFP expression**	2. Cells are analyzed under a fluorescent microscope or Flow Cytometry (FACS) to confirm the GFP or RFP activity level. • For example, every 24 hrs after CRISPR plasmids treatment, prepare the sample for fluorescent microscope and FACS. • Confirm the GFP expression over time and proceed to the next step.	
3	 **Isolation and expanding monoclonal cell colonies**	3. 2-3 days after CRISPR plasmids treatment, plate appropriate cell density to isolate monoclonal cell colonies. • The colony formation efficiency could vary among cell lines. Thus, an optimal density of the cell population needs to be determined empirically. - Dish method: plate 50, 250, 1,000, and 5,000 cells/100 mm dish (2 plates/cell population) - Limiting dilution method: plate 0.4 cell/well of 96-well plate (2-3 plates) (It is recommended to proceed with the test for each cell, which means screening after colony seeding.) ✓ <u>Save some of the cell population treated with CRISPR plasmids, and confirm the efficient mutation induction by CRISPR plasmids in your target cell by a T7E1 assay.</u> 4. Isolate and expand monoclonal cell colonies after 10-20 days of plating (50-100 colonies are recommended).	
4	**Identification of the knock-out cell clones**		
	 **Genotyping**	5. Prepare genomic DNA from each clone between a 48-well plate and a 12-well plate (the plate wells may change depending on the purpose of the experiment). 6. Identify the knock-out cell clones by genotyping. (In/del analysis by NGS or/and T7E1 assay screening.) • T7E1 assay screening: Screening of isolated colonies by T7E1 assay will identify cell clones with a mutation at the target site but will not be able to discern the heterozygous and homozygous knockout cell. Refer to the Mutation Detection Kit Manual for additional information regarding the T7E1 assay screening.	• Most mutation induced by CRISPR plasmids at the target site is small deletions and insertions (-20 to +10 bp). When these mutations are causing the frameshift, it will function as a knockout mutation. • Cell lines usually contain more than 2 alleles (polyploidy). • The complete knockout cell lines will have frameshift mutation on all alleles of the target gene.
	 **Immunoblot analysis**	5. Prepare protein from each clone between a 48-well plate and a 12-well plate (the plate wells may change depending on the purpose of the experiment). 6. Identify the knock-out cell clones by immunoblot analysis.	

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