AccuTool™ pRGEN_CjCas9_CMV/T7

[Cat. No.] ATS-0102, ATS-0103

Introduction

CjCas9 is derived from *Campylobacter jejuni* (*C. jejuni*) and is the smallest Cas9 orthologue (2.9 kb), which improves the disadvantages of large-sized SpCas9 (4 kb), derived from *Streptococcus pyogenes* (*S. pyogenes*).
The CjCas9 gene can be packaged in an adeno-associated virus (AAV) vector together with a reporter gene and is highly specific *in vitro* and *in vivo*, enabling precise genome editing applicable for treatment.
DNA-directed RNA-Guided Endonucleases (dRGENs) are efficient, affordable, and convenient tools for genome editing experiments. *AccuTool*™ pRGEN_CjCas9_CMV/T7, CjCas9 expression plasmid can be used with custom CjCas9-sgRNA expression plasmid (dRGEN-CjCas9), which uses the CMV/T7 promoter for strong transient expression of CjCas9. Plasmids can be delivered to your cell of interest by any standard methods like lipofection, nanoparticle, or electroporation to achieve highly efficient delivery.

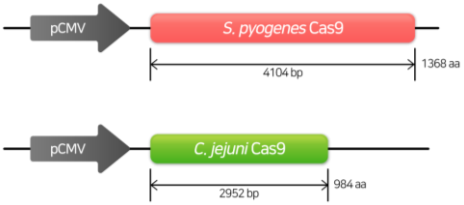


Figure 1. Cas9 orthologues

Applications

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

Components

Components	Amount
Lyophilized pRGEN_CjCas9 Plasmid	5 μg or 50 μg

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

Specifications

Expression vectors for CjCas9 gene (pRGEN_CjCas9_CMV/T7) are ampicillin-resistance and stable in general *E. coli* strains such as DH5α or XL1.

Figure 2. pRGEN_CjCas9_CMV/T7

Storage

- *AccuTool*™ pRGEN_CjCas9_CMV/T7 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.

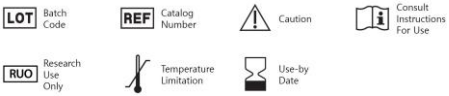
Ordering Information

Description	Cat. No.
AccuTool™ pRGEN_CjCas9_CMV/T7 - 5 μg	ATS-0102
AccuTool™ pRGEN_CjCas9_CMV/T7 - 50 μg	ATS-0103

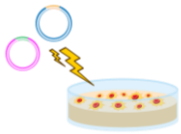
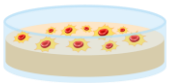
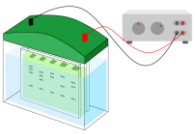
Notice

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



Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th><th></th></tr>
<tr><td colspan="4">Gene knock-out cell establishment</td></tr>
<tr><td>1</td><td> 
Cell transfection</td><td colspan="2">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.</td></tr>
<tr><td>2</td><td> 
Isolation and expanding monoclonal cell colonies</td><td colspan="2">2. 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.)
✓ 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.
3. Isolate and expand monoclonal cell colonies after 10-20 days of plating (50-100 colonies are recommended).</td></tr>
<tr><td rowspan="3">3</td><td colspan="3">Identification of the knock-out cell clones</td></tr>
<tr><td> 
Genotyping</td><td>4. 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).
5. Identify the knock-out cell clones by genotyping.
(In/del analysis by NGS or/and T7E1 assay screening.)
• T7EI 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.</td><td rowspan="2">• 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.</td></tr>
<tr><td> 
Immunoblot analysis</td><td>4. 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).
5. Identify the knock-out cell clones by immunoblot analysis.</td></tr>
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