AccuTool™ sgRNA-(GFP) synthesis (dRGEN) (V0/2021-07-14)

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

Introduction

DNA-directed RNA-Guided Endonucleases (dRGENs) are efficient, affordable, and convenient tools for genome editing experiments. *AccuTool*™ dRGEN recognizes a target sequence that is 23 bp in length and ends with two guanines (GG). Custom sgRNA expression plasmids can be used with Cas9 expression plasmids (human codon optimized, WT/ Nickase /Sniper form available). Plasmids can be delivered to your cell of interest by any standard methods like lipofection, nanoparticle, or electroporation to achieve highly efficient delivery. sgRNA-GFP expression plasmids are constructed by inserting a GFP construct in the existing sgRNA plasmids. sgRNA-GFP expression plasmids allow you to confirm the activity level in cells by fluorescent microscope. The *AccuTool*™ dRGEN is a custom-designed sgRNA, which is provided as a sgRNA expression plasmid or sgRNA-GFP expression plasmid.

Applications

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

Components

Components	Amount
Lyophilized sgRNA-(GFP) Plasmid (dRGEN)	2 μg or 50 μg

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

Specifications

Expression vectors for guide RNAs (dRGEN-U6-sgRNA and dRGEN-U6-sgRNA:GFP-CMV) are ampicillin-resistance and stable in general *E. coli* strains such as DH5α or XL1.

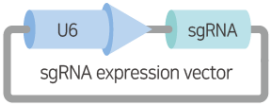


Figure 1. dRGEN-U6-sgRNA

Figure 2. dRGEN-U6-sgRNA:GFP-CMV

Storage

- *AccuTool*™ dRGEN 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.

sgRNA Information

Name	Target Sequence

Online Resources

Korean English

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

Ordering Information

Description	Cat. No.
AccuTool™ sgRNA synthesis (dRGEN) - 2 μg	ATC-0050
AccuTool™ sgRNA synthesis (dRGEN) - 50 μg	ATC-0051
AccuTool™ sgRNA synthesis (dRGEN:GFP-CMV) - 2 μg	ATC-0052
AccuTool™ sgRNA synthesis (dRGEN:GFP-CMV) - 50 μg	ATC-0053

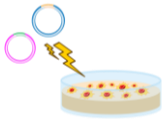
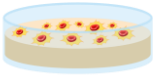
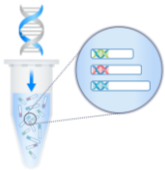
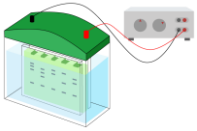
Notice

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

REF Catalog Number | Caution | Consult Instructions For Use | RUO Research Use Only

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th colspan="2">Procedure Details</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> 
(Optional) Detection of GFP expression</td><td colspan="2">2. Cells are analyzed under a fluorescent microscope or Flow Cytometry (FACS) to confirm the GFP 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.</td></tr>
<tr><td>3</td><td> 
Isolation and expanding monoclonal cell colonies</td><td colspan="2">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).</td></tr>
<tr><td rowspan="3">4</td><td colspan="3">Identification of the knock-out cell clones</td></tr>
<tr><td> 
Genotyping</td><td>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.</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>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.</td></tr>
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