AccuTool™ Positive control (dRGEN) (V0/2021-07-14)

[Cat. No.] ATS-0054, ATS-0055, ATS-0056

Introduction

DNA-directed RNA-Guided Endonucleases (dRGENs) are efficient, affordable, and convenient tools for genome editing experiments. *AccuTool*™ dRGENs positive controls are validated guide RNA sequences provided in plasmids cloned into sgRNA expression plasmids, and they are verified to be highly genome editing efficient. Positive control 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.

Applications

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

Components

Components	Amount
Lyophilized sgRNA Plasmid (dRGEN)	2 μg
Lyophilized Forward Primer	1 nmol
Lyophilized Reverse Primer	1 nmol

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

Specifications

Expression vector for guide RNAs (dRGEN-U6-sgRNA) is ampicillin-resistance and stable in general *E. coli* strains such as DH5α or XL1.

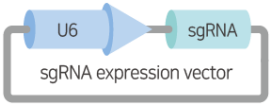


Figure 1. dRGEN-U6-sgRNA

Storage

- *AccuTool*™ dRGENs positive controls are 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.

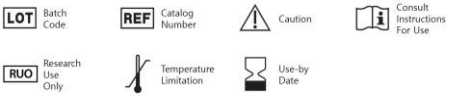
Ordering Information

Description	Cat. No.
AccuTool™ Positive control_EGFP sgRNA (dRGEN)	ATS-0054
AccuTool™ Positive control_CCR5 sgRNA (dRGEN)	ATS-0055
AccuTool™ Positive control_HPRT1 sgRNA (dRGEN)	ATS-0056

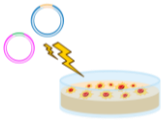
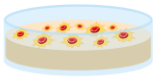
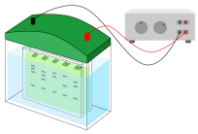
Notice

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 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> 
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.)
✓ <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>
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.)
• 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>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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