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

[Cat. No.] ATS-0006, ATS-0007, ATS-0008

Introduction

Active RNA-Guided Endonucleases (aRGENs) are ready-to-use guide RNAs for your genome editing experiments. *AccuTool*™ aRGENs positive controls have validated guide RNA sequences, and they are verified to be highly genome editing efficient. When combined with suitable Cas9 mRNA (optional) or recombinant Cas9 protein (optional), aRGENs will form ready-for-action to genome engineering. The reagents can be used for *in vitro* and *in vivo* without additional processing.

Applications

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

Components

Components	Amount
Lyophilized sgRNA (aRGEN)	2 nmol
Lyophilized Forward primer	1 nmol
Lyophilized Reverse primer	1 nmol
DEPC-treated water	1 ml

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

Reconstitution of lyophilized sgRNA

- Dissolve lyophilized guide RNA in DEPC-treated water.
- Recommended concentration of stock guide RNA solution.

For animal embryo injection	1-2 μg/μl
For *in vitro* digestion study	1-2 μg/μl
For genome engineering in cultured cells	5 μg/μl

Storage

- *AccuTool*™ aRGENs positive controls are lyophilized and delivered at ambient temperature.
- Store lyophilized RNAs or reconstituted RNA stock solutions at or below -20°C. Do not store in a frost-free freezer.

Precautions

- As RNA oligonucleotides (aRGENs) are highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free conditions.
- RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

Online Resources

Korean

English

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

Ordering Information

Description	Cat. No.
AccuTool™ Positive control_EGFP sgRNA (aRGEN)	ATS-0006
AccuTool™ Positive control_CCR5 sgRNA (aRGEN)	ATS-0007
AccuTool™ Positive control_HPRT1 sgRNA (aRGEN)	ATS-0008

Notice

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Explanation of Symbols

Use-by Date

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Experimental Procedures

Steps		Procedure Details
Digestion of target sequence *in vitro* using CRISPR sgRNAs		
1	**Preparation of reaction mixture**	1. Set up the reaction mixture as below.
2	**Incubation of reaction mixture**	2. Incubate the reaction mixture at 37°C for 1 hr. 3. Add 4 μg of RNase and incubate for 15 min at 37°C. 4. Add 1 μl of STOP solution to the reaction mixture and incubate for 15 min at 37°C. [STOP solution: 30% glycerol, 1.2% SDS, 250 mM EDTA (pH 8.0)] 5. Analyze on 2% agarose gel.

Reaction mixture (Step 1):

Components	Amount	
Cas9 Protein	500 ng	(100-1,000 ng)
CRISPR sgRNA	250 ng	(100-700 ng)
Targeting substrate	100-150 ng	PCR product
	80 ng	(Plasmid)
10X Reaction buffer	1 μl	
D.W.	To 10 μl	

General guidelines for the application of CRISPR RNP to cultured cells by lipid-based transfection (Lipofection)

- The amounts of the reagents given in the protocol below are for one well of a 24-well plate. For other reaction formats, scale the amounts of reagents up or down accordingly.

Steps		Procedure Details
1	**Preparation of transfection reagent**	1. Add Cas9 RNP complex (0.5 μg of Cas9 Nuclease and 250 ng of CRISPR sgRNA) to 50 μl Opti-MEM I Reduce Serum Medium. 2. In a separate tube, dilute the transfection reagent by adding 4 μl of the Lipofectamine 2000 transfection reagent to 50 μl of Opti-MEM I Reduce Serum Medium and mix gently. 3. Incubate for 5 min at room temperature. 4. Add the diluted transfection reagent to the tube containing Cas9 protein/gRNA RNP complexes and mix gently. 5. Incubate at room temperature for 20 min to allow the formation of Cas9/gRNA-lipid complexes.
2	**Cell transfection**	6. Add the Cas9/gRNA-lipid complexes to the $1x10^5$ NIH3T3 cells to be transfected. Swirl the plates gently to allow the mixing of the transfection mixture with the medium. 7. Incubate the plate at 37°C in a humidified CO_2 incubator in a cell culture incubator for 2-3 days, and proceed with sample assay to determine the genome editing efficiency by T7E1 assay or targeted deep sequencing.
	Option	Refer to the electroporation method for highly efficient transfection only.

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