GenCrispr sgRNA Screening Kit

Cat. No. L00689 **Version 02142017**

I DESCRIPTION

Cas9 Nuclease NLS, *S. pyogenes,* is an RNA-guided endonuclease that catalyzes site-specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif). The design of single guide RNA (sgRNA) is dependent on the target region close to the PAM site. Even if you pick a target sequence that fulfills all of the described requirements, sgRNA specificity and activity is unpredictable. Therefore, it is often recommended that multiple, different sgRNAs be designed to target a gene of interest. The GenCrispr sgRNA Screening Kit provides a simple, reliable, and rapid method for assessing sgRNA efficiency before cell transduction, allowing you to identify the highly effective CRISPR sgRNA.

II KIT CONTENTS

Components	Amount	
	30-reaction kit	100-reaction kit
GenCrispr Cas9 Nuclease	15 µL	50 µL
10X Reaction Buffer	100 µL	300 µL
Positive Control sgRNA	3 µg	10 µg
Positive Control Substrate	60 µL	200 µL
RNase-free water	1 mL	1 mL

Materials required to prepare:

The following materials need to be prepared by clients:

1. Experimental sgRNAs containing the specific sequence complementary to target DNA: It can be synthesized directly or transcribed by RNA polymerase.
2. DNA substrates containing the target DNA sequence. Please follow the protocol below.

III APPLICATIONS

Evaluate the efficiency of sgRNAs for CRISPR/Cas9 *in vitro* studies and identify the highly effective sgRNAs before cell transfection.

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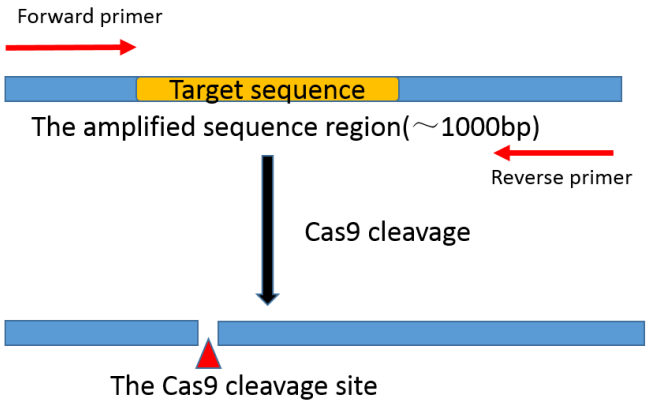
IV STORAGE

1. The positive control sgRNA is provided as a freeze-dried powder that should be stored at -80°C once received. It is recommended to be used within 3 months after receiving. Redissolve with RNase-free water before using.
2. The activity of the positive control sgRNA will decrease after free-thaw cycles. Please aliquot and store at -80°C, to avoid repeated freeze/thaw cycles.
3. The recommended storage temperature of GenCrispr Cas9 Nuclease and 10X Reaction buffer is -20 °C.

V PROTOCOL

1. Prepare experimental DNA substrate

Design primers to amplify the sequence region targeted by CRISPR/Cas9. The optimal amplicon size is ~1000bp, the cleavage fragments of which are suggested to be of different sizes with the minimum size being at least 250bp after Cas9 cleavage. The amplification substrate can be linearized plasmids, PCR products, or synthesized oligonucleotides. The final PCR product is taken as the experimental DNA substrate for cleavage assays, but should be gel purified if non-specific bands are present.



2. Set up a cleavage reaction containing your experimental sgRNA sample and your experimental DNA substrate, in parallel with a positive control reaction.

Reagents	Experimental cleavage reaction	Positive control cleavage reaction
Experimental sgRNA	100—500 ng	----
Positive Control sgRNA	---	1 μL (100 ng)
GenCrispr Cas9 Nuclease	~0.25 μL（50 ng）	~0.25 μL (50 ng)
10X Reaction Buffer	2 μL	2 μL
RNase-free water	0--17 μL	Up to 18 μL
Incubate the above mixture for 10 min at 37℃.		
Experimental DNA substrate	~160 ng	----
Positive control substrate	----	2 μL
Total volume per reaction	20 μL	20 μL

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3. Mix gently and Incubate at 37℃ for 2 hours.
4. Analyze 10 µL reactions on a 1% agarose gel alongside a negative control (~100 ng of Experimental DNA substrate). The control substrate is predicted to be partially cleaved into 1053 bp and 526 bp respectively.

NOTES

1. The cleavage efficiency of sgRNA and Cas9 *in vitro* studies may not be exactly the same as the activity of *in-vivo* studies.
2. Research purposes only. This product may not be used for any other purposes, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans.

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