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sgRNA Screening Kit

Catalog #: NB-GEN10642 (96 wells)

User Manual

This kit is designed to provide a simple, reliable, and rapid in vitro method for assessing sgRNA efficiency before cell transformation, allowing the identification of the highly effective CRISPR sgRNAs.

Manufactured and Distributed by:

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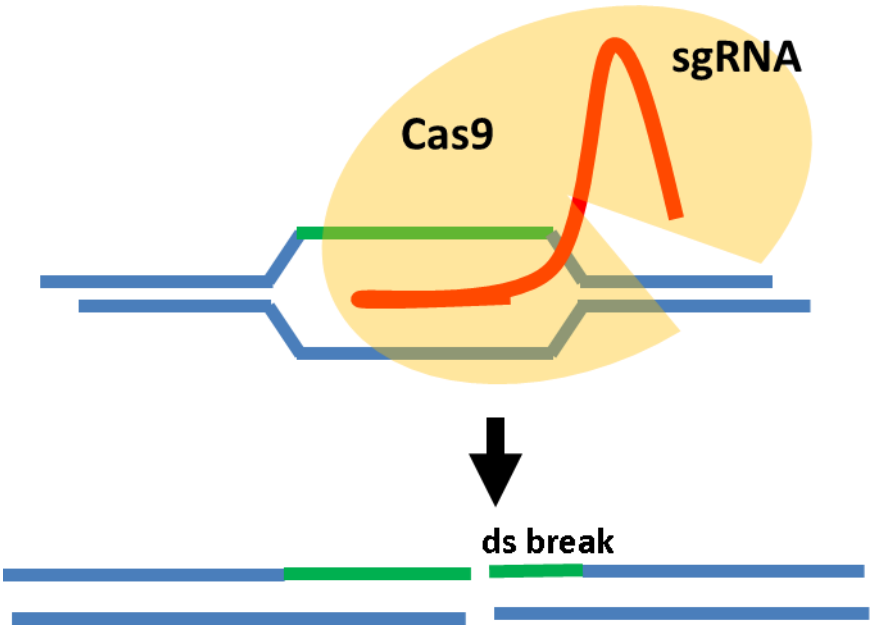
Description

Cas9 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 the sgRNA sequence fulfills all of the described requirements, its specificity and activity is unpredictable. Therefore, it is often recommended to design multiple sgRNAs to target a gene of interest. This kit is designed to provide a simple, reliable, and rapid in vitro method for assessing single guide RNA (sgRNA) efficiency before cell transformation, allowing the identification of the highly effective CRISPR sgRNA from a pool of sgRNAs.

Assay time	120 min
Validity	Six months
Store at	-80°C

Assay principle



Materials Supplied

Components	Amount
Cas9 Nuclease	50 µL
10X Reaction Buffer	1 mL
Positive Control sgRNA	10 µL
Positive Control Substrate	40 µL
RNase-free water	1 mL

Materials required but not supplied

- Experimental sgRNAs containing the specific sequence complementary to target DNA (directly synthesized or transcribed by RNA polymerase).
- DNA substrate containing the target DNA sequence.

Creating a Cleavage Template

Design primers to amplify your target sequence within a ~2 kb amplicon, with the sgRNA target sequence located asymmetrically within the amplicon. This will enable the cleavage reaction with the Cas9-sgRNA complex to generate two fragments of unequal size (differing by more than 500 bp) that are clearly separated on an agarose gel.

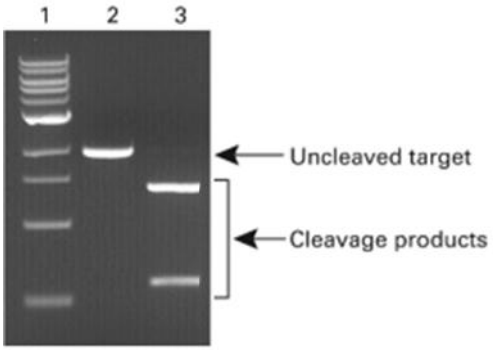
Setting up Cas9 reaction

Reagents	Experimental reaction	Positive control reaction
Experimental sgRNA	100—500 ng	----
Positive Control sgRNA	---	1 µL (100 ng)
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

Mix gently and Incubate at 37℃ for 1.5 hours.

Analyze 10 µL reactions on a 1% agarose gel alongside a negative control (~100 ng of Experimental DNA substrate).

The positive control is predicted to be partially cleaved into 1053 bp and 526 bp respectively.



Analysis of cleavage products. Positive control cleavage reaction according to the above protocol. Agarose gel analysis indicated that 100 ng sgRNA (Lane 3) is sufficient for 100% cleavage of the target fragment. Lane 2 shows the untreated/uncleaved control