CRISPR sgRNA Screening Kit (#BN00128)

(Cat# BN00128; 25 Reactions; Store at -20°C)

I. Introduction:

Assay Genie's CRISPR sgRNA Screening Kit (*S. pyogenes*) is a very handy and efficient tool for selecting the most promising sgRNA design to be used with the spCas9 system. sgRNA sequences can be synthesized using our Classic or Express CRISPR sgRNA Synthesis Kit (Cat. No. #BN00129 and #BN00130 respectively). The target template for screening will be PCR-generated using the 2X PCR MasterMix included in this kit. The sgRNA screening conditions have been optimized and the sgRNA cleavage reaction can be completed within one hour. *In vitro* screening and selecting the most efficient sgRNA design can greatly reduce the time and labor investment in any downstream CRISPR genome editing applications.

II. Application:

- Screening of sgRNA

III. Kit Contents (25 rxns):

Components	Quantity	Part Number
Cas9 Nuclease NLS Protein	50 µl	BN00128-1
10X Cas9 Reaction Buffer	1.25 ml	BN00128-2
Control Primers & Template	10 µl	BN00128-3
Protein Degrader	150 µl	BN00128-4
Control sgRNA	5 µl	BN00128-5
Cell Lysis Buffer	1.25 ml	BN00128-6
2X PCR Mix	1 ml	BN00128-7
Nuclease-free H_2O	1 ml	BN00128-8

IV. Shipment and Storage:

All the reagents are shipped at -20°C. Store all components at -20°C in a non-defrost cycle freezer. All the components are stable for 2 years from the date of shipment when stored and handled properly.

V. Protocol:

Part I. Cleavage Template Amplification:

The cleavage template is generated by PCR amplification of the gene of interest from gDNA. The target fragment should be at least 2 kb in length. Primers should be designed such that the cleavage site does not lie at the midpoint of the amplicon in order to produce two distinguishable product bands. It is recommended that the two product bands are more than 500bp different in size for the ease of gel analysis. or high GC content templates, include 5-10% DMSO in the PCR reaction. The reagents included in this kit allow for simple amplification of the target fragment from genomic DNA of the "wildtype" cells.

1. Add 4 µl of Protein Degrader and 50 µl of Cell Lysis Buffer to the desired cell pellet containing $5x10^4$-$2x10^6$ cells. After fully resuspending the cells, incubate the mixture at 58°C for 30 min, followed by 95°C for 10 min.
2. Set up the PCR immediately following lysis. Otherwise, store the cell lysate at -20 °C.
3. Thaw 2X PCR MasterMix and primers on ice. Mix solutions thoroughly. Set up the following reactions in sterile PCR tubes on ice. Mix well and centrifuge briefly.

Product Components	Control	Positive	Negative	Final Concentration
Control Primer & Template	1 µl	-	-	1X
Cell Lysate from step 1	-	2 µl	-	Variable
Primer Mix	-	1 µl	1 µl	200-500 nM
2X PCR Mix	25 µl	25 µl	25 µl	1X
Nuclease-free H_2O	24 µl	22 µl	24 µl	-
Total Volume			**50 µl**	

4. Perform PCR amplification as follows:

Product Components	Temperature	Time	Cycle (s)
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	30 sec	40
Annealing	45-72°C (60°C for control)	30 sec	
Extension	72°C	3.5 kb/min (1 min for control)	
Final Extension	72°C	5 min	1
Hold	4°C	Hold	1

5. To check PCR amplification, load 7-10 µl of PCR product on a 1% agarose gel. Proceed to next step when a clean product band of desired size is obtained, with no significant non-specific amplification.

Part II. Screening:

1. Setup the screening reaction as follow:

Product Components	Quantity
10X Cas9 Reaction Buffer	2 µl
Cas9 Nuclease NLS Protein	2 µl
Synthesized Target-Specific* or Control sgRNA	1 µl
Incubate for 15 mins at 37°C, then add the following	
Cleavage Template from Part I, step 5	5-15 µl
Nuclease-free H_2O	0-10 µl
Total Volume	**20 µl**

2. Incubate the reaction at 37°C for 30 mins, then add 2 µl of Protein Degrader to each reaction followed by another incubation at 37°C for 10 mins. Degrading the Cas9 protein will terminate the reaction and will yield a much cleaner pattern in the subsequent gel analysis.
3. Analyze the cleavage on a 1% agarose gel. A 1.5kb and a 0.6kb band are produced by the cleavage of the control template with the control sgRNA.