CRISPR Genomic Cleavage Detection Kit

(Cat# BN00127; 25 Reactions; Store at -20°C)

I. Introduction: Precision is a key quality in genome editing and gene modification methods. Confirmation of successful gene editing is an important step in any set up, and could save both time and resources if experimental insufficiencies are caught early on. The **CRISPR Genomic Cleavage Detection Kit** is designed as an easy and yet effective way to verify your genomic editing process. CRISPR-edited cell samples are used as a template in PCR reactions targeting the specific region of interest. The products are then denatured and re-annealed to produce mismatches in the double strand. These mismatches are recognized and subsequently cleaved by the detection enzyme to produce product sizes that are easily distinguishable upon gel analysis. Assay Genie's ready-to-use **CRISPR Genomic Cleavage Detection Kit** provides a simple, rapid assay and thus will surely be an indispensable addition to any genomic-editing toolbox.

II. Application:

- Genome Editing

III. Key Features:

- Ease of use with simple steps
- Rapid set-up
- Streamlined protocol suitable for high-throughput applications

IV. Kit Contents:

Components	Quantity	Part Number
Cell Lysis Buffer	1.25 ml	BN00127-1
Protein Degrader	50 µl	BN00127-2
Detection Enzyme	13 µl	BN00127-3
10X Detection Buffer	50 µl	BN00127-4
Control Primer & Template	10 µl	BN00127-5
Distant^{TM} PCR Mix (#M1136-200)	1 ml	BN00127-6
Nuclease-free H_2O	1 ml	BN00127-7

V. 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.

VI. Protocol:

Cell Lysis:

a) Prepare cell pellets to contain $5x10^4$-$2x10^6$ cells.
b) Add 2 µl of Protein Degrader to 50 µl Cell Lysis Buffer in an Eppendorf tube. Mix well.
c) Add 50 µl of Protein Degrader/Cell Lysis Buffer mixture to cell pellet to resuspend.
d) Incubate tube at 68°C for 15 min, followed by 95°C for 10 min.
e) Start PCR amplification step immediately following lysis. Otherwise, store lysates at -20°C.

PCR Amplification:

DNA sample preparation, reaction set-up and subsequent reactions should be performed in separate areas to avoid cross-contamination. Please note that primers should be designed such that the cleavage site is not in the middle of the amplicon, so the detection reaction will produce two distinguishable product bands. For high GC content reactions, include 5-10% DMSO.

a) Thaw 2X PCR Mix and primers on ice. Mix solutions thoroughly.
b) Set up the following reactions in sterile PCR tubes on ice. Mix well and centrifuge briefly.

Component	Control	Positive	Negative	Final Concentration
Control Primer & Template	1 µl	-	-	1X
Cell Lysate	-	2 µl	-	Variable
Primer Mix	-	1 µl	1 µl	200 nM
2X PCR Mix	25 µl	25 µl	25 µl	1X
Nuclease-free H_2O	24 µl	22 µl	24 µl	-

c) Perform PCR amplification as follows:

Step	Temperature	Time	Cycle (s)
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	30 sec	40
Annealing	45 - 72°C (55°C for control)	30 sec	
Extension	72°C	3.5kb/min (1min for control)	
Final Extension	72°C	5 min	1
Hold	4°C	Hold	1

d) To check PCR amplification, load 3 µl of PCR product on a 1% gel. Proceed to next step when a clean product band of desired size is obtained, with no significant non-specific amplification.

Cleavage Assay:

a) Set up the following reactions in sterile PCR tubes on ice. Mix well and centrifuge briefly.

Component	Reaction Volume
PCR Product	1-6 µl
10X Detection Buffer	1 µl
Nuclease-free H_2O	up to 9.5 µl

b) Perform re-annealing reaction with the following cycle:

Stage	Temperature	Time	Temperature/Time
1	95°C	5 min	-
2	95-85°C	-	-2°C/sec
3	85-255°C	-	0.1°C/sec

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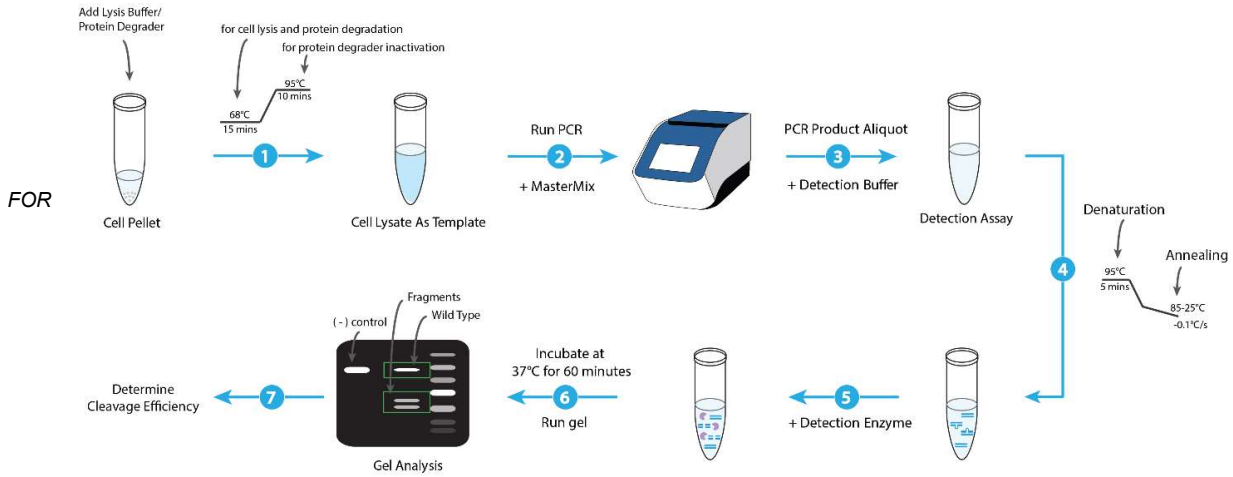
4	4°C	Hold	-

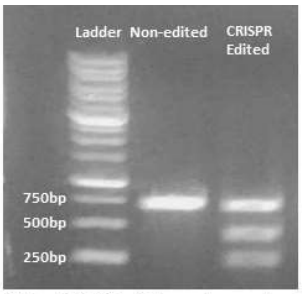
Gel Analysis:

a) Add 0.5 µl of Detection Enzyme to all samples containing positive and control reactions. Mix well, centrifuge briefly.
b) Incubate at 37°C for 1 hour.
c) Vortex samples, spin down. Load 10 µl of each sample with DNA ladder and loading dye on a 1-2% agarose gel at low voltage for ≈ 30 min.
d) View on a UV transilluminator, obtain gel picture using a gel imaging system.
e) Using gel analysis software, determine the relative amount of DNA contained in each band
f) Calculate cleavage efficiency using the following equation:

Cleavage efficiency = 1-(1-fraction cleaved)1/2

VII. Workflow Summary:





Both non-edited and CRISPR-edited samples were treated with the CRISPR Genomic Cleavage Detection Kit. CRISPR-edited samples in the right lane show expected band sizes with approximately 430bp and 260bp after enzymatic cleavage. Non-edited samples in the left lane did not show enzymatic cleavage after treatment.

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