



CRISPR Genomic Cleavage Detection Kit

Cat. No. G932

Store at -20°C

Product Description

The **CRISPR Genomic Cleavage Detection Kit** is designed as a simple, rapid assay 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. Included in the kit is a control template with the size of 750 bp after PCR amplification and of 500 bp and 250 bp after the cleavage assay.

Product Component	Quantity	Part No.
Cell Lysis Buffer	1.25 ml	G932-1
Protein Degradar	50 µl	G932-2
Detection Enzyme	13 µl	G932-3
10X Detection Buffer	100 µl	G932-4
Control Primer & Template	10 µl	G932-5
MegaFi™ Pro Fidelity 2X PCR MasterMix	1.25 ml	P887-1
Nuclease-Free H ₂ O	1.0 ml	P100

Protocol

Cell Lysis

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

PCR Amplification

Note: 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.

1. Thaw MegaFi™ Pro Fidelity 2X PCR MasterMix and primers on ice. Mix solutions thoroughly.

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

Component	Control	Positive	Negative	Final Conc.
Control Primer & Template	1 µl	-	-	1X
Cell Lysate	-	2 µl	-	variable
Primer Mix	-	1 µl	1 µl	200 nM
MegaFi™ Pro Fidelity 2X PCR MM	25 µl	25 µl	25 µl	1X
Nuclease-Free H ₂ O	24 µl	22 µl	24 µl	-

3. Perform PCR amplification as follows:

Step	Temperature	Time	Cycle(s)
Enzyme Activation	95°C	5 min	1
Denaturation	95°C	15 sec	40
Annealing	variable 55°C for control	15 sec	
Extension	72°C	3 kb/min 15 sec for control	
Final Extension	72°C	5 min	1
Hold	4°C	Hold	1

4. 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

1. 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	2 µl
Nuclease-Free H ₂ O	up to 19.5 µl

2. 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	85-25°C	-	-0.1°C/sec
4	4°C	Hold	-

3. Add 0.5 µl of Detection Enzyme to all samples containing positive and control reactions. Mix well, centrifuge briefly.
4. Incubate at 37°C for 1 hour. Vortex samples, spin down.
5. Load samples on an appropriate agarose gel and obtain gel picture using a gel imaging system.
6. Analyze the cleavage efficiency using the following equation:

$$\text{Cleavage efficiency} = 1 - (1 - \text{fraction cleaved})^{1/2}$$