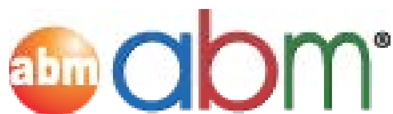

CRISPR Genomic Cleavage Detection Kit

Store at –20°C

Product	Quantity	Cat. No./Part No.
Cell Lysis Buffer	1.25 ml	G932-1
Protein Degrader	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™ Fidelity 2X PCR MasterMix	1.25 ml	P897-1
Nuclease-Free H_2O	1.0 ml	P100

Product Description

Genome editing and gene modification methods are widely used and precision of these techniques is a necessity. Confirmation of successful gene editing is an important step, saving downstream resources if nonperformances are caught early. 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.

Storage

Store all components at -20°C in a non-frost-free freezer. All components are stable for 2 years from the date of shipping when stored and handled properly.

Protocol

Cell Lysis

1. Prepare cell pellets to contain 5×10^4 - 2×10^6 cells.
2. Add 2 µl of Protein Degrader to 50 µl Cell Lysis Buffer in an Eppendorf tube. Mix well.
3. Add 50 µl of Protein Degrader/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

DNA sample preparation, reaction set-up and subsequent reactions should be performed in separate areas to avoid cross-contamination. 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.

1. Thaw MegaFi™ 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™ Fidelity 2X PCR MasterMix	25 µl	25 µl	25 µl	1X
Nuclease-Free H_2O	24 µl	22 µl	24 µl	-

3. Perform PCR amplification as follows:

Step	Temperature	Time	Cycle(s)
Enzyme Activation	98°C	2 min	1
Denaturation	98°C	10 sec	40
Annealing	55°C	30 sec	
Extension	72°C	20 sec	
Final Extension	72°C	2 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_2O	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	-

Gel Analysis

1. Add 0.5 µl of Detection Enzyme to all samples containing positive and control reactions. Mix well, centrifuge briefly.
2. Incubate at 37°C for 1 hour.
3. Vortex samples, spin down. Load 15 µl of each sample with DNA ladder and loading dye on a 1-2% agarose gel at low voltage for approximately 30 min.
4. View on a UV transilluminator, obtain gel picture using a gel imaging system.
5. Using gel analysis software, determine the relative amount of DNA contained in each band.
6. Calculate cleavage efficiency using the following equation:

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