Tribioscience

CRISPR Genomic Cleavage Detection Kit

Catalog	Unit
TBS6096	100 Reactions

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.

Component

Product Component	Quantity
Cell Lysis Buffer	1.25 ml
Protein Degrader	100 µl
Detection Enzyme	50 µl
10X Detection Buffer	200 µl
Control Primer & Template	10 µl
Pro Fidelity 2X PCR MasterMix	2 x 1.25 ml

Additional Materials Required
- Target-Specific Primers
- CRISPR-Edited Cells
- Wild-Type Cells
- Nuclease-Free H_2O

Store at -20°C.

Protocol

Part A – Cell Lysis

1. Pellet CRISPR-Edited and Wild-Type cells when confluency reaches 70-90% in a 96-well plate (approximately 1-4 × 104 cells) and discard supernatant.
2. In a new eppendorf tube, mix the following:

Product Component	Volume per Cell Pellet
Protein Degrader	1 µl
Cell Lysis Buffer	10 µl
Nuclease-Free H_2O	39 µl

3. Transfer 50 µl of the mixture into each cell pellet and gently resuspend cells.
4. Incubate at 68°C for 15 minutes followed by inactivation at 95°C for 10 minutes. The lysates can be used directly as PCR templates.

Part B – PCR

1. Set up the following reactions:

Product Component	Control	Samples
Control Primer & Template	1 µl	-
Cell Lysate	-	2 µl
Primer Mix	-	1 µl
Pro Fidelity 2X PCR MM	12.5 µl	12.5 µl
Nuclease-Free H_2O	11.5 µl	9.5 µl

2. Perform PCR amplification as follows:

Step	Temperature	Time	Cycle
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	35
Annealing[1]	55°C for control	15 sec	
Extension[1]	72°C	15 sec (for control) 2-3 kb/min	
Final Extension	72°C	5 min	1

[1]Adjust annealing temperature and extension time for your Target-Specific Primers.

3. Run 5 µl of each product on a 2% agarose gel. Confirm correct size of Target-Specific amplicon (variable) and each Control amplicon (expected size is 750 bp). Keep the remaining PCR products on ice or at -20°C until Part C. If non-specific amplifications appear, repeat the PCR using touchdown method.

Part C – Cleavage Assay

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

Product Component	Volume per Cell Pellet
PCR Product	1-6 µl
10X Detection Buffer	2 µl
Nuclease-free Water	Up to 19.5 µl

1. Perform re-annealing reaction with the following cycle:

Temperature	Time	Ramping Rate
95°C	5 min	-
95°C-85°C	-	-2°C/sec
85°C-25°C	-	-0.1°C/sec
4°C	Hold	-

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

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

General Notes

- 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. (ex. 500 bp and 250 bp after cleavage)
- For a more accurate readout, PCR amplicons used in the assay should not contain non-specific amplifications and primer dimers.
- This kit is intended to only detect the presence of editing by employing T7 Endonuclease I which recognizes mismatching brought by a mixture of edited and unedited pools. For monoclonal selection of CRISPR Knock-Out cells, use CRISPR Cas9 Cleavage Detection Kit (Cat. TBS6097).

For research use only