GenCrispr Mutation Detection Kit

Cat. No. L00688 **Version 02142017**

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I DESCRIPTION

The GenCrispr Mutation Detection Kit provides a simple, reliable, and rapid method for the detection of site specific cleavage of genomic DNA that is extracted from cells transfected with constructs expressing engineered nucleases such as Transcription activator-like effector nucleases (TALEN), Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9, or Zinc-finger nucleases (ZFN). The GenCrispr Mutation Detection Kit includes High-Fidelity DNA polymerase for amplifying the target regions from cells, and GenCrispr T7 Endonuclease I for recognizing and detecting the mismatches caused by gene editing tools. It provides an easy and reliable approach for estimating the efficiency of genome editing.

II KIT CONTENTS

Components	Amount provided	
	25-reaction kit	100-reaction kit
High-Fidelity DNA polymerase	10 µL	40 µL
5X PCR Reaction Buffer	150 µL	600 µL
GenCrispr T7 Endonuclease I (Cat.No.Z03396)	25 µL	100 µL
10X GenCrispr T7 Endonuclease I Reaction buffer	50 µL	500 µL
Control Template DNA	30 µL	120 µL
Control Primer Mix	30 µL	120 µL
Protease K	25 µL	100 µL
dNTP	15 µL	60 µL

III APPLICATIONS

Detect the gene mutagenesis and SNP, for cleavage efficiency assay caused by ZFNs, TALENs, CRISPR/Cas9 or other gene editing tools.

IV STORAGE

Store all components at -20°C upon receipt.

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V PROTOCOL

Harvest cells:

1. Spin down cells transfected with TALEN, CRISPR, or ZFN constructs at 12000 rpm for 1 minute at 4°C.
2. Carefully remove supernatant, and proceed to the following steps or store the cell pellets at –80°C.

Samples Prepared for PCR amplification:

Use a genomic DNA extraction kit to extract genomic DNA from harvested cells, or lyse the cell pellets directly with cell lysis buffer (e.g. QuickExtract DNA Solution from Epicenter) for the following genomic cleavage detection assay.

PCR amplification:

Set up a 25 μl PCR reaction using ~100 ng of genomic DNA as a template. Add the following components to PCR tubes.

Content	Samples	Control
5X PCR Reaction Buffer	5 μL	5 μL
10 uM dNTP mixture	0.5 μL	0.5 μL
10 uM Primer F/R Mix	1 μL	---
Control primer mix	---	1 μL
Template (~100 ng)	~100 ng	1 μL
High-Fidelity DNA polymerase	0.25 μL	0.25 μL
Nuclease-free water	Up to 25 μL	Up to 25 μL

Run a PCR reaction according to the following program:

Steps	Temperature	Time
Initial Denaturation	98 ℃	30 seconds
25—35 Cycles	98 ℃	5—10 seconds
	*50—72 ℃	10—30 seconds
	72 ℃	30—40 seconds/kb
Final Extension	72 ℃	2 minutes
Hold	4—10 ℃	

Note: Thermocycling conditions for the positive control are as follows: 98 ℃ / 10 s, 60 ℃ / 15 s, 72 ℃ / 15 s, for 35 cycles.

Supplements: If non-specific bands are present, PCR reactions should be purified by gel extraction prior to further fragment analysis.

Heteroduplex formation:

Assemble the reaction as follows:

Reagent	Amount
PCR reaction	10 μL
10x Reaction buffer	2 μL
Nuclease-free water	Up to 19 μL

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Denature and then anneal the products in a thermocycler using the following program:

Cycle steps	Temperature	Ramp Rate	Time
Initial Denaturation	95 ℃		5 min
Annealing	95-85 ℃ 85-25 ℃	-2 ℃ /second -0.1 ℃/second	
Hold	4 ℃		

Alternatively, if a thermocycler is not available with these ramp speeds, the samples can be heated to 95ºC for 10 minutes and then allowed to cool to room temperature gradually.

Heteroduplex digestion:

Reagent	Amount
Annealed PCR product	19 μL
T7 Endonuclease I	1 μL

Mix well and briefly spin. Incubate each reaction at 37 ℃ for 15 minutes. Detect it directly by agarose gel electrophoresis or add 1 μL protease K and incubate for at 37 ℃ for 5 minutes to stop the reaction.

Detection

Add loading buffer to the reaction mixture directly, and detect the cleavage efficiency by agarose gel electrophoresis. (The size of the positive control PCR product is 589 bp, which will be partially cleaved into 341 bp and 248 bp fragments)

NOTE

Research purposes only. This product may not be used for any other purposes, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans.

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