# GenCrispr Mutation Detection Kit

Cat. No.: L00688 Version 2023-08-25

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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.




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# II Contents

The components of GenCrispr Mutation Detection Kit are described in the table below.

| Components | 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 | 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 Application

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.

# V Protocol

## Harvest cells:

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



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## 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 DNA as a template. Add the following components to PCR tubes.

| Content | Samples | Control |
|---|---|---|
| 5X PCR Reaction Buffer | 5 µL | 5 µL |
| 10 µM dNTP mixture | 0.5 µL | 0.5 µL |
| 10 µM 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 °C | 30 seconds |
| 25-35 Cycles | 98 °C | 5-10 seconds |
| | *50-72 °C | 10-30 seconds |
| | 72 °C | 30-40 seconds/kb |
| Final Extension | 72 °C | 2 minutes |
| | 4-10 °C | Hold |

Note: Thermocycling conditions for the positive control are as follows: 98 °C / 10 s, 60 °C / 15 s, 72 °C / 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.**



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## 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 |

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

Assemble the reaction as follows:

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

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 °C for 15 minutes. Detect it directly by agarose gel electrophoresis or add 1 µL protease K and incubate for at 37 °C for 5 minutes to stop the reaction.



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### 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.

## VI Ordering Information

| Product Name | Cat. No. |
|---|---|
| GenCrispr T7 Endonuclease I | Z03396 |

## Contact us

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