AccuCRISPR™ Mutation Detection Kit (T7E1) (V3/2025-12-05)

[Cat. No.] ATS-0125

Introduction

AccuCRISPR™ Mutation Detection Kit [T7 Endonuclease Ⅰ (T7E1)] can detect on-target genome editing and evaluate their efficiency. This method has the advantage of being able to analyze quickly and simply.
In the T7E1 assay, targeted genomic regions are amplified by PCR and the PCR products are denatured and reannealed to produce heteroduplex DNA. T7E1 recognizes heteroduplex DNA and cleaves at the first, second, or third phosphodiester bond that is 5´ to the mismatch. The results can be analyzed by agarose gel electrophoresis. It is a reliable method to measure genome editing efficiency through the gel band's intensity and obtain consistent data.

Applications

- Genome editing efficiency analysis and SNP detection: This can be confirmed by cutting the mismatched part of the heteroduplex DNA and checking the band.
- Used for error correction method of artificial gene synthesis.

Components

Cat. No.	ATS-0125
T7 Endonuclease Ⅰ (T7E1)	250 U (10,000 U/ml)
10X T7E1 reaction buffer	1 ml
Positive control (PCR product)	1 μg (lyophilized form)

* **Note:** For research use only. Not for use in diagnostic or therapeutic procedures.

Buffer Composition

1X T7E1 Reaction buffer	50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9

Storage Buffer

T7 Endonuclease Ⅰ is supplied in 1X storage buffer (50 mM Tris-Cl (pH 7.6), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.05% NaN_3, 50% glycerol).

Unit Definition

One unit is defined as the amount of enzyme required to convert > 90% of 1 μg of supercoiled cruciform pBHA (AT) to > 90% linear form in a total reaction volume of 50 μl in 1 hour at 37°C.

Quality Control

Activity Test: Add 1 μl T7E1 to 200 ng positive control (PCR product) of heteroduplex DNA and react at 37°C for 15 minutes. Add 6X DNA loading buffer to the reaction mixture and proceed with agarose gel electrophoresis to check the cut band.

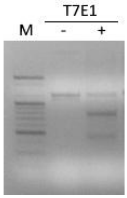


Figure1. T7E1 assay of control PCR product

Storage

- *AccuCRISPR*™ Mutation Detection Kit components are delivered at -20°C.
- The recommended storage temperature is -20°C and avoid repeated thawing and freezing of T7E1 as this may affect the performance.

Online Resources

Korean English

Visit our **product page** for additional information and protocols.

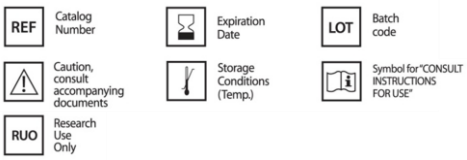
Ordering Information

Description	Cat. No
AccuCRISPR™ Mutation Detection Kit (T7E1)	ATS-0125

Notice

Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

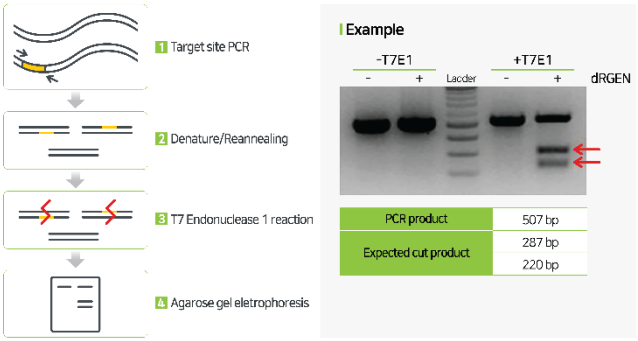
Explanation of Symbols



Experimental Procedures

Steps	Procedure Details

T7 Endonuclease I recognizes mismatched DNA, heteroduplex DNA, and T7 Endonuclease I cleaves at the first, second, or third phosphodiester bond that is 5' to the mismatch.



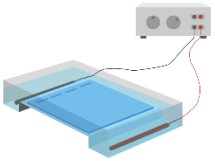
1

Genomic DNA Preparation

1. Prepare CRISPR-Cas9 edited genomic DNA.

2. PCR amplifies the genomic DNA purified from transfected CRISPR plasmids.
   - Set up a PCR reaction using up to 100 ng of genomic DNA as a template. The amplified PCR product is usually around 500 bp, and the target site is better to avoid the middle of the PCR product.

2



T7 Endonuclease I Digestion & Detection

3. Heteroduplex formation: The PCR products must be denatured and annealed in order to allow formation of heteroduplex between PCR products with and without mutations.
   - Prepare reaction mixture, and then denature and anneal the PCR products under the following conditions.

*** Note:** Positive control for the T7E1 assay should also be prepared by heteroduplex form.

Components	Amount
PCR products	200 ng
10X T7E1 buffer	2 µl
D.W.	Up to 19 µl

Denature and annealing condition	
95°C	5 min
95°C to 85°C	-2°C/sec
85°C to 25°C	-0.1°C/sec
4°C	Hold

4. Add 1 µl of T7E1 to the reaction mixture, and incubate at 37°C for 15 minutes.
   *** Note:** Incubation above 42°C causes an increase in non-specific nuclease activity and should be avoided.

5. Detect the digestions on 2% agarose gel.