T7 Endonuclease I

Catalog #	3610	3612
Package Size	500 units	1,250 units
Concentration	10 units/µl	

Description

T7 Endonuclease I, a stable homodimer of identical 149 amino acid subunits (1) is the product of a recombinant gene in E. coli . Double-stranded breaks (DSBs) generated by CRISPR/TALEN at desired target sites can be PCR-amplified, and the PCR products can be denatured and re-annealed to form mismatched DNA. If the mismatched DNA length position is more than 1 bp, T7 endonuclease I can recognize and cleave it. It is useful for quantitatively estimating the nuclease-induced mutation frequency of gene edited cells.

Protein Purity

The physical purity of this enzyme is ≥99% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

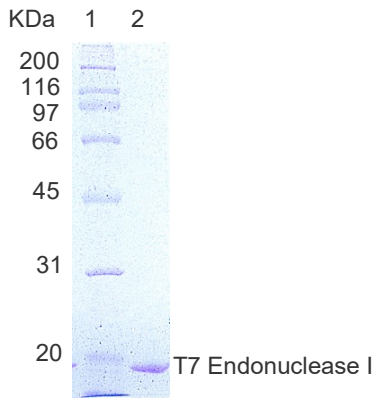

Figure 1: Lane 1. Protein marker
Lane 2. T7 Endonuclease

Product Source

E. coli BL21 (DE3) pLysS strain expressing T7 Endonuclease I gene.

Applications

- Resolve four-way junction or branched DNA
- Detect or cleave heteroduplex and nicked DNA
- Randomly cleave linear DNA for shot-gun cloning

Product Includes

- T7 Endonuclease I
- 10x T7 Endonuclease I reaction buffer

Storage Temperature

-20 ºC

Storage Buffer

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25ºC

1x T7 Endonuclease I Reaction Buffer

10 mM Tris-HCL, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.5 @ 25 ºC

Quality Control Assays

T7 Endonuclease I is free from detectable contaminating nuclease activities.

Protocol

This protocol describes how to determine genome targeting efficiency by digesting annealed mismatched PCR products with T7 Endonuclease I. In the first step, PCR products are produced from the genomic DNA of cells whose genomes were targeted using Cas9, TALEN, ZFN etc. In the second step, the PCR products are annealed and digested with T7 Endonuclease I. If two shorter fragments of the expected size are generated, which means that it has successfully introduced mutations small insertions and deletions (indels typically 2~20 bp) are present at the targeted chromosomal site. Fragments are analyzed to determine the efficiency of genome targeting.

Overview:

Cell lysis
↓
Genomic DNA extraction (Optional)
↓
Target site PCR amplification
↓
DNA purification (Optional)
↓
Denaturation and re-annealing
↓
T7 Endonuclease I digestion

Related Products

- Cas9 Nuclease (Cat.# 3273)
- T4 DNA Ligase (Cat.# 3212)
- Taq DNA Polymerase (Cat.# 3243)
- Taq DNA Polymerase 2x Premix (Cat.# 3249)

Technical Support

Intact Genomics is committed to supporting the world-wide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.
Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

T7 Endonuclease I

Intact Genomics®

Procedures:

I. Sample Preparation

- **Option 1:** Genomic DNA extraction
  Harvest cells and extract genomic DNA according to the manufacturer extraction protocol.
- **Option 2:** Cell lysate preparation
  Collect cells and add 50-100 µl Lysis buffer and lyse cells at 95 ºC for 5-10 min.

II. PCR Amplification

1. Thaw the kit components, mix and pulse-spin in microfuge each component prior to use.
2. Set up a 50 µl PCR reaction using up to 100-200 ng of genomic DNA as a template. Assemble the following reaction:

Reagent	50 µl reaction
Genomic DNA	100-200 ng
Forward primer (10 µM)	2.5 µl
Reverse primer (10 µM)	2.5 µl
2x PCR Master Mix	25.0 µl
H_2O up to	50.0 µl

3. Mix the reaction and transfer the tubes to a PCR machine with the following thermocycling conditions:

PCR Cycling Conditions			
Steps	**Temp.**	**Time**	**Cycles**
Initial Denaturation	95 ºC	5 min	1
Denaturation	95 ºC	30 sec	30
Annealing	Tm-3 ºC	30 sec	
Extension	72 ºC	1 min	
Final Extension	72 ºC	5 min	1

4. Run 3-5 µl of the PCR product on a 1-2% agarose gel. The final PCR product should be within the range of 600 – 1000 bp.
5. *(Optional)* If necessary, purify the DNA by using either gel extraction kit or ampure XP beads according to the manufacturer protocol.

III. Hybridization (Denaturation and Reannealing)

1. Assemble reactions as follows:

Component	50 µl reaction
DNA (wt)	15.0 µl
DNA (mutant)	15.0 µl
10x Endonuclease I buffer	5.0 µl
H_2O	15.0 µl
Total	50.0 µl

2. Mix the reaction gently.
3. Heat at 95 ºC for 5 min by using a heat block. Thermocycler can also be used for hybridization.
4. Turn off the heat block and cool down gradually to room temperature.

IV. T7 Endonuclease I Digestion

1. Assemble reactions as follows:

Hybridized DNA	5.0 µl
10x Endonuclease I buffer	1.0 µl
T7 Endonuclease I (10 U)	1.0 µl
H_2O up to	10.0 µl

2. Incubate at 37 ºC for 15-30 min.
3. Stop the reaction by adding 1.0 µl of 0.5 M EDTA.
4. Run 1-2% agarose gel to see the cleavage efficiency. A typical gel for Endonuclease I (Endo I) cleavage is shown below:

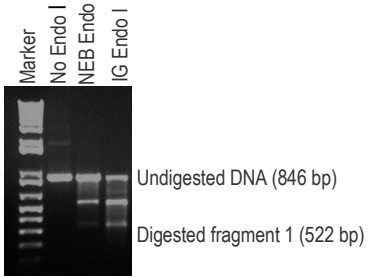

Figure 2: T7 Endonuclease I activity testing: Double-stranded (ds) DNA fragments containing two types of sequences are PCR amplified, denatured and annealed to produce mismatched (heterologous) dsDNA. Purified mismatched dsDNA digested with Endonuclease I from NEB and Intact Genomics (IG) to compare the enzyme efficiency.

V. Troubleshooting

Problems	Possible Causes	Recommendations
Non-specific cleavage	•Non-specific PCR amplification	•Purify DNA •Optimize PCR primers •Optimize PCR conditions
No cleavage	•Low T7 Endonuclease I activity	•Add more T7 Endo I
No expected bands	•Incomplete lysis •Poor PCR primer	•Adjust the volume of lysis buffer according to the cell number •Increase the lysis time •Optimize PCR primers