GenCrispr T7 Endonuclease I
Cat. No. Z03396 **Version 08102016**

I DESCRIPTION

T7 Endonuclease I (T7E1) recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions, hetero duplex DNA and more slowly, nicked double-stranded DNA. The cleavage site is at the first, second or third phosphodiester bond that is 5´ to the mismatch. The protein is the product of T7 gene 3. GenCrispr T7 Endonuclease I is a fusion protein produced from *E.coli.*

II KIT CONTENTS

Kit Contents	Quantity	Catalog No.	Components/Concentration
GenCrispr T7 Endonuclease I	250 U	Z03396-250	10000 U/ml
	1250 U	Z03396-1250	10000 U/ml
10X Reaction Buffer	1ml		500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM DTT, pH 7.9 , 25 °C

III APPLICATIONS

- **Resolve four-way junction or branched DNA**
- **Detect or cleave hetero duplex and nicked DNA**
- **Randomly cleave linear DNA for shot-gun cloning**
- **Detect gene mutagenesis and SNPs, for cleavage efficiency assays induced by TALEN, CRISPR/CAS9 or other gene editing tools.**

IV QUALITY CONTROL ANALYSIS

- **High Protein purity:** GenCrispr T7 Endonuclease I is > 95% pure as determined by SDS-PAGE using Coomassie Blue detection.
- **High Bioactivity**: > 90% of 1 µg of supercoiled cruciform pUC (AT) to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C. The target DNA substrate can be cleaved and detected with 15 min at 37 °C.

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V UNIT DEFINITION

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

Note: pUC (AT) is derived from pUC19 with a modification of the polylinker between the EcoRI site and PstI site.

EcoRI PstI

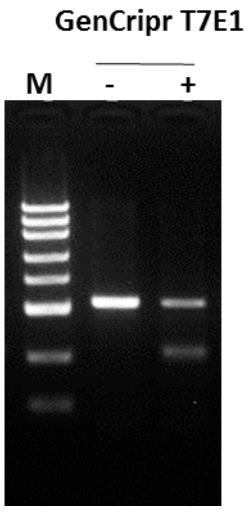
GAATTCATATATATATATATATATATATATATATATATATATATATCTGCAG

VI STORAGE

GenCrispr T7 Endonuclease I is supplied in 1X storage buffer (200 mM NaCl,20 mM Tris-HCl (pH 7.5) , 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100 and 50% glycerol). The recommended storage temperature is -20°C.

VII ACTIVITY TEST

To test the activity of GenCrispr T7 Endonuclease I, GenScript used a control gRNA targeting HPRT, which is co-transfected with Cas9 protein into 293T cells. After 48 h, cells were lysed for genome PCR to amplify the specific target site. The PCR product (~200 ng) was then annealed and incubated with 1 µl T7E1 for 15 min at 37 °C. Add loading buffer to the reaction mixture directly, and detect the cleavage efficiency by agarose gel electrophoresis.



The T7E1 assay to detect the cleavage efficiency of control HPRT gRNA with Cas9 protein.

VIII PROTOCOL

PCR:

Set up a 50 µl PCR reaction using ~100 ng of genomic DNA as a template. For each amplicon set up 2 PCR reactions using the following templates:

gDNA from targeted cells (e.g. Cas9, or TALEN transfected cells)

gDNA from negative control cells (e.g. non-specific DNA transfected cells)

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The amplification fragment is usually around 500 bp and the target site is better to avoid the middle of fragment.

T7 Endonuclease I digestion:

Assemble reactions as follows:

PCR Products	200 ng
10 X reaction buffer	2 µl
Nuclease-free Water	To 19 µl

Anneal the above PCR products in a thermocycler using the following conditions:

Initial Denaturation	
95°C	5 minutes
Annealing	
95-85°C	-2°C/second
85-25°C	-0.1°C/second
Hold	4°C

Add 1µl T7 Endonuclease I to the annealed PCR products

Incubation Time 15 minutes at 37°C

Detection

Add loading buffer to the reaction mixture directly, and detect the cleavage efficiency by agarose gel electrophoresis.

Note

T7 Endonuclease I is a structure-selective enzyme. It acts on a variety of DNA substrates with different specific activities. It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate. Temperatures above 42°C cause an increase in nonspecific nuclease activity and should be avoided.

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