UltraPrecise T7 Endonuclease I

Catalogue number: MB34001, 50 reactions
MB34002, 200 reactions

Description

UltraPrecise T7 Endonuclease I is a proprietary enzyme developed to recognise and cleave incorrect impairments of double DNA strands caused by errors during the gene synthesis process. Insertion, deletions or mismatch mutations can be easily removed from synthetic DNA using an error correction step, which includes the UltraPrecise T7 Endonuclease I. The addition of this additional error correction step in gene synthesis workflows allows to increase the probability of isolating a synthetic gene with a correct sequence by 4-8-fold. Thus, we suggest to include an error removal step after PCR assembly or amplification (Figure 1) in order to decrease the error rate of your gene synthesis protocol.

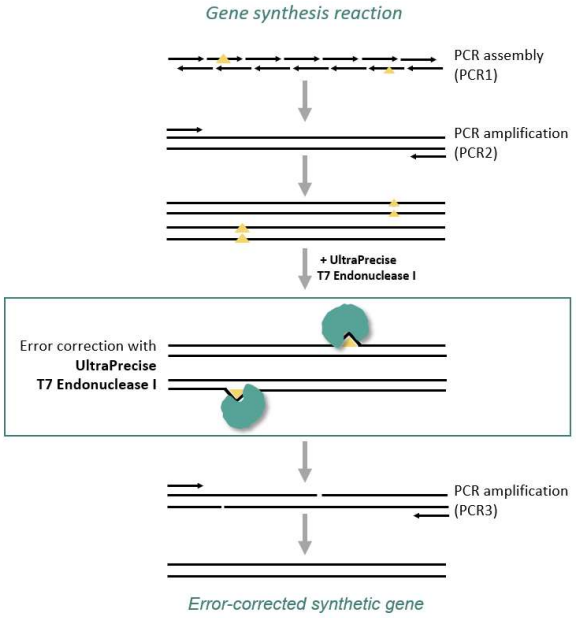

Figure 1. Gene synthesis workflow. The error correction step using UltraPrecise T7 Endonuclease I is highlighted with the green box. This step should be performed after a denaturing-reannealing step that follows gene synthesis, which creates the DNA mismatches used by UltraPrecise T7 Endonuclease I as substrate.

Storage temperature

UltraPrecise T7 Endonuclease should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Inactivation

The enzyme is heat inactivated at 65 °C for 20 min.

System components and Reaction conditions

UltraPrecise T7 Endonuclease I is provided with a dedicated and highly optimized NZYtech reaction buffer and displays an optimum temperature of 25 °C.

Standard protocol for *in vitro* error-correction

The following standard protocol serves as a general guideline for any error correction reaction using the UltraPrecise T7 Endonuclease I. We recommend quantifying the PCR product using a nanodrop or other accurate methodology.

1. DNA dilution

Dilute the PCR product to 25 ng/µL in 1× reaction buffer for UltraPrecise T7 Endonuclease I as following:

Component	Volume
10× Reaction buffer (provided)	5 µL
Template DNA	x
Nuclease-free H_2O (Cat. No. MB11101)	up to 20 µL

2. Denaturation/re-annealing of DNA strands to create DNA mismatches

Denature and re-anneal the diluted PCR products in a thermocycler using the following conditions:

Temperature	Time
95 °C	2 min
4 °C	5 min
37 °C	5 min
4 °C	∞

3. Error correction reaction

3.1. Add 1 µL of UltraPrecise T7 Endonuclease I to 10 µL of the re-annealed PCR product.

3.2. Incubate the error correction reaction at 25 °C for 30 minutes. After the incubation, immediately place the reaction on ice.

3.3. Add 1 µL of 50 mM EDTA (not provided) to slow the UltraPrecise T7 Endonuclease I activity.

3.4. Immediately proceed with the final PCR amplification in order to recover the error-corrected DNA fragments.

Quality Control Assays

Purity

UltraPrecise T7 Endonuclease is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by BlueSafe staining (NZYtech, Cat. No. MB15201).

Nucleases assays

To test for DNase contamination, 0.2-0.3 µg of supercoiled pNZY28 plasmid DNA are incubated with 1 µL of UltraPrecise T7 Endonuclease for 14-16 hours at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with 1 µL of UltraPrecise T7 Endonuclease for 1 hour at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible cutting of the nucleic acids.

Functional assay

UltraPrecise T7 Endonuclease I is tested for activity in a standard gene synthesis reaction with error-correction. Efficiency of enzyme's reaction is analyzed through DNA sequencing.

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Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.: +351.213643514 Fax: +351.217151168
www.nzytech.com