NZY Thermolabile Uracil-DNA Glycosylase

Catalogue number: MB44501, 100 µL
MB44502, 3 x 100 µL

Description

NZY Thermolabile Uracil-DNA Glycosylase is an engineered version of an Uracil-DNA Glycosylase (UDG) from a cold-adapted organism that, at low temperatures (20-25°C), catalyses the release of free uracil from uracil-containing DNA templates. The enzyme efficiently hydrolyses uracil from single-stranded or double-stranded DNA (although it is more active on ssDNA), but not from RNA. The enzyme excises uracil, thus creating alkali-sensitive abasic sites in the DNA. These abasic sites can be hydrolysed by heat and thus the contaminant dU-containing LAMP/PCR products from previous experiments can no longer serve as amplification templates. Since the enzyme is inactivated at temperatures of 45°C or higher, NZY Thermolabile Uracil-DNA Glycosylase is useful in a variety of applications where carry-over contamination can be a problem. These include PCR/qPCR or LAMP (since the LAMP reaction takes place at 65°C), as well as these reactions merged with reverse transcription (RT) – RT-LAMP or RT-PCR/qPCR (given that RT usually takes place at 45-55°C). At 45°C, NZY Thermolabile Uracil-DNA Glycosylase is completely inactivated and thus will not target the newly generated uracil-containing cDNA/DNA chains in RT or LAMP/PCR reactions, respectively. An incubation step with NZY Thermolabile Uracil-DNA Glycosylase for 2-5 min at 25°C before PCR/qPCR reactions is all that is required to prevent the amplification of contaminant LAMP/PCR products carried over.

Shipping Conditions

The product can be shipped in a range of temperatures from dry ice to blue ice.

Storage Conditions

All components should be stored between -30°C and -15°C in a constant temperature freezer. Avoid multiple freeze-thaw cycles to guarantee maximal shelf life. The product will remain stable until the expiry date if stored as specified.

Pack components

Component	MB44501	MB44502
NZY Thermolabile Uracil-DNA Glycosylase (5 U/µL)	100 µL	3 x 100 µL
Reaction buffer, 10×	2 x 1000 µL	4 x 1000 µL

Unit definition

One unit is defined as the amount of enzyme that catalyses the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA, at 25°C in controlled assay conditions.

Enzyme concentration 5 U/µL, in glycerol.

Standard Protocols

NZY Thermolabile Uracil-DNA Glycosylase is active over a broad pH range with an optimum at pH 8.0. No cofactors or divalent cations are required for activity. The enzyme is functional in most conventional LAMP, RT-LAMP, PCR/qPCR or RT-PCR/qPCR reaction buffers. For the direct application of NZY Thermolabile Uracil-DNA Glycosylase in uracil containing LAMP, RT-LAMP, PCR/qPCR or RT-PCR/qPCR master mixes, add 1 µL of NZY Thermolabile Uracil-DNA Glycosylase per 50 µL reaction. Incubate at 25°C for 2-5 minutes. The enzyme is inactivated at 45°C for 10 minutes. Alternatively, the following standard protocol serves as a general guideline and a starting point for usage of NZY Thermolabile Uracil-DNA Glycosylase in other applications:

1. Gently mix and briefly centrifuge all components after thawing. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a mixture for the appropriate number of reactions. Add water first and the remaining components in the order specified in the table below. A single reaction mixture of 50 µL should combine the following components:

Reaction buffer, 10×	5 µL
Uracil-containing DNA (PCR product)	<0.1 µg
NZY Thermolabile Uracil-DNA Glycosylase (5 U/µL)	0,5 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reaction.

3. Incubate at 25°C for 2-5 minutes. Time can be extended whenever there is a change in the standard set up described above that may benefit from longer incubation periods.

Inactivation

NZY Thermolabile Uracil-DNA Glycosylase is inactivated at 45°C for 10 minutes in standard conditions.

Uracil-containing DNA template

The DNA template for NZY Thermolabile Uracil-DNA Glycosylase activity should contain uracil in a significant proportion. Uracil-containing DNA can be prepared by *in vitro* methods, in particular through PCR reactions containing a proportion or the totality of dTTP replaced by dUTP. Prior to the usage of NZY Thermolabile Uracil-DNA Glycosylase, uracil-containing DNA fragments should be checked to confirm that all DNA molecules contain uracil (only those can act as a substrate for the enzyme). In case a proportion of DNA fragments do not contain uracil, then those will remain as potential PCR/qPCR contaminants for subsequent reactions.

Quality control assays

Purity

NZY Thermolabile Uracil-DNA Glycosylase purity is >90% as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie Blue staining.

Genomic DNA contamination

NZY Thermolabile Uracil-DNA Glycosylase must be free of any detectable genomic DNA contamination as evaluated through PCR.

Nuclease assays

Typically, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of NZY Thermolabile Uracil-DNA Glycosylase, in 1× Reaction Buffer, for 14-16 hours at 37°C. To test for RNase contamination, 1 µg of RNA is incubated with 5 U of NZY Thermolabile Uracil-DNA Glycosylase, in 1× Reaction Buffer for 1 h at 37°C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with the reaction buffer.

Functional Assay

NZY Thermolabile Uracil-DNA Glycosylase activity is measured in a carryover prevention experiment using approximately 10^3 copies of a DNA fragment containing dU prior to the amplification reaction by real-time PCR. After treatment with NZY Thermolabile Uracil-DNA Glycosylase, under standard conditions, no amplification products are detected.

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