NZY Uracil-DNA Glycosylase

Catalogue number: MB44601, 100 µL
MB44602, 3 x 100 µL

Description

NZY Uracil-DNA Glycosylase is an engineered version of a psychrophilic Uracil-DNA Glycosylase (UDG) that catalyses the release of free uracil from uracil-containing DNA templates. The enzyme is optimized for removal of potentially carry-over contaminant uracil-containing LAMP or PCR products in LAMP or PCR/qPCR scenarios, respectively. NZY Uracil-DNA Glycosylase 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 55°C or higher, NZY Uracil-DNA Glycosylase is recommended for removal of carry-over contaminants exclusively in LAMP or PCR/qPCR reactions. Thus, NZY Uracil-DNA Glycosylase is not recommended for removal of carry-over contaminants from RT-LAMP or RT-PCR/qPCR reactions that include a reversion transcription step at 45-55°C. At these temperatures NZY Uracil-DNA Glycosylase remains partially active and thus will degrade the newly generated uracil-containing cDNA chains.

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	MB44601	MB44602
NZY 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 catalyzes 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 Uracil-DNA Glycosylase is active over a broad pH range with an optimum at pH 8.0. The enzyme is functional in most conventional LAMP or PCR/qPCR reaction buffers. For the direct application of NZY Uracil-DNA Glycosylase in uracil containing LAMP or PCR/qPCR master mixes, add 1 µL of NZY Uracil-DNA Glycosylase per 50 µL reaction. Incubate at 25°C for 2-5 minutes and heat inactivate at 65°C or 95°C for 2-5 min, in LAMP or PCR/qPCR, respectively. Usage of a "HotStart" Taq DNA polymerase is strictly required in these master mixes, due to significant levels of polymerase activity at 25°C. Alternatively, the following standard protocol serves as a general guideline and a starting point for usage of NZY 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 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 Uracil-DNA Glycosylase is inactivated at 55°C for 10 minutes in standard conditions.

Uracil-containing DNA template

The DNA template for NZY 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 in reactions containing a proportion or the totality of dTTP replaced by dUTP. Previous to the usage of NZY 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 those will remain as potentially PCR/qPCR contaminants for subsequent reactions.

Quality control assays

Purity

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

Genomic DNA contamination

NZY 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 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 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 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 Uracil-DNA Glycosylase under standard conditions, no amplification products are detected.

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For research use only.