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T4 β-Glucosyltransferase

Catalog Number: E100-1, E100-2

Table 1. Kit Components and Storage

Kit Component	E100-1 (500 units)	E100-2 (2500 units)	Storage	Stability
T4 β-Glucosyltransferase (10 units/µL)	50 µL	250 µL	-20 °C, avoid repeated freeze-thaw	The product is stable for at least 6 months when stored as directed.
10× Reaction Buffer	250 µL	1.25 mL		

Product Description

T4 β-glucosyltransferase (T4 BGT) is a DNA-modifying enzyme encoded by bacteriophage T4 that catalyzes the transfer of glucose (Glc) from uridine diphosphoglucose (UDP-Glc) to 5-hydroxymethylcytosine (5-hmC) residues in double-stranded DNA, resulting in the formation of β-glucosyl-5- hydroxymethylcytosine.

The enzyme is available in 500 and 2,500 unit sizes at a concentration of 10 U/µL. The enzyme is supplied with a 10× Reaction Buffer.

Applications

- Glucosylation or immunodetection of 5-hmC DNA.
- Differentiation of 5-hmC from 5-mC.
- 5-hmC containing DNA enrichment.
- Labeling of 5-hmC residues using a radioactive UDP-glucose donor.

Product Specifications

- **Storage Buffer:** 50 mM Tris-HCl (pH 7.5 at 25 °C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, and 50% (v/v) glycerol.
- **Unit Definition:** One unit is defined as the amount of enzyme required to protect 0.5 µg T4gt-DNA against cleavage by MfeI restriction endonuclease.
- **Protection Unit Assay Conditions:** 0.5 µg T4gt-DNA, 1X reaction buffer and 40 µM UDP-Glucose in a 30 µL reaction. Incubate for 1 hour at 37°C followed by 10 minutes at 65°C. The extent of protection by T4 β-glucosyltransferase is determined by the addition of 20 µL 1X reaction buffer and 10 units of MfeI. Incubation at 37°C for 30 minutes is followed by analysis on agarose gels.

General Protocol

1. Assemble the following reaction at room temperature:

10× Reaction Buffer	5 µL
2 mM UDP-Glucose	5 µL
DNA	up to 1 µg
Nuclease-free water	to 49 µL
T4 BGT	1 µL
Total volume	50 µL

2. Mix gently and spin down for a few seconds.
3. Incubate at 37 °C for 1 hour.
4. Stop the reaction by heating at 65 °C for 20 min.