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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 free-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.

## Application

- 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.