**T4 Phage β-glucosyltransferase (T4-BGT)**

**M0357S**

500 units 10,000 U/ml Lot: 0041612
RECOMBINANT Store at −20°C Exp: 12/18

**Description:** T4 Phage β-glucosyltransferase specifically transfers the glucose moiety of uridine diphosphoglucose (UDP-Glc) to the 5-hydroxymethylcytosine (5-hmC) residues in double-stranded DNA, making beta-glucosyl-5-hydroxy-5-methylcytosine (5-hmC) residues in double-stranded DNA, making beta-glucosyl-5-hydroxy-5-methylcytosine (1,2).

**Source:** An *E. coli* strain that carries the cloned bgt gene from bacteriophage T4.

Supplied in: 20 mM KPO4, 200 mM NaCl (pH 7.0 @ 25°C), 0.1 mM EDTA, 0.25 mM dithiothreitol and 50% glycerol.

**Applications:**
- Glucosylation of 5-hydroxymethylcytosine in DNA (1)
- Immunodetection of 5-hydroxymethylcytosine in DNA (3)
- Labeling of 5-hydroxymethylcytosine residues by incorporation of [H]- or [14C]-glucose into 5-hmC-containing DNA acceptor after incubation with [H]- or [14C]-UDP-Glc (4).
- Detection of 5-hydroxymethylcytosine in DNA by protection from endonuclease cleavage.*
  * The sensitivities of restriction endonucleases to DNA modifications, including glucosylated hydroxyethylcytosine are catalogued on REBASE (http://rebase.neb.com/rebase/rebmss.html).

**Reagents Supplied with Enzyme:**
10X NEBuffer 4, 50X UDP-Glucose (2 mM).

**Reaction Conditions:** 1X NEBuffer 4 and 40 µM UDP-Glucose. Incubate at 37°C.

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**1X NEBuffer 4:**
- 50 mM potassium acetate
- 20 mM Tris-acetate
- 10 mM magnesium acetate
- 1 mM dithiothreitol
- pH 7.9 @ 25°C

**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 NEBuffer 4 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 -BGT is determined by the addition of 20 µl 1X NEBuffer 4 and 10 units of MfeI. Incubation at 37°C for 30 minutes is followed by analysis on agarose gels.

**Diluent Compatibility:** Diluent Buffer B 300 mM NaCl, 10 mM Tris-Cl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

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**Quality Control Assays**

**Exonuclease Assays:** Incubation of a 50 µl reaction containing 100 units of T4 -BGT with 10 pmol of a mixture of single and double-stranded [H]-E. coli DNA (10⁵ cpm/µg) for 4 hours at 37°C resulted in <0.1% of the total radioactivity.

**16-Hour Incubation:**
A 50 µl reaction containing 1 µg of DNA and 100 units of T4-BGT for 16 hours at 37°C resulted in a DNA pattern free of detectable nucleic acid degradation as determined by agarose gel electrophoresis.

**Endonuclease Assay:**
Incubation of a 50 µl reaction containing 100 units of T4-BGT with 1 µg of qX174 DNA for 4 hours at 37°C resulted in <10% conversion to RFI as determined by agarose gel electrophoresis.

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Enzyme Properties

Activity in NEBuffers:
NEBuffer 1 100
NEBuffer 2 50
NEBuffer 3 50
NEBuffer 4 100%

Survival in a Reaction: A minimum of 0.16 unit for 16 hours is required to protect 0.5 µg T4 gt-DNA against cleavage by MfeI.

Heat Inactivation: 65°C for 10 minutes

Molecular Weight: 40,666 kDa

References:

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