

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



1-800-632-7799  
info@neb.com  
www.neb.com



M0357S 005170319031

**M0357S**



**500 units      10,000 U/ml      Lot: 0051703**

**RECOMBINANT    Store at -20°C    Exp: 3/19**

**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-hydroxymethylcytosine (1,2).

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

Supplied in: 20 mM KPO<sub>4</sub>, 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 [<sup>3</sup>H]- or [<sup>14</sup>C]-glucose into 5-hmC-containing DNA acceptor after incubation with [<sup>3</sup>H]- or [<sup>14</sup>C]-UDP-Glc (4).
- Detection of 5-hydroxymethylcytosine in DNA by protection from endonuclease cleavage.\*

\* The sensitivities of restriction endonucleases to DNA modifications, including glucosylated hydroxymethylcytosine are catalogued on REBASE (<http://rebase.neb.com/rebase/rebms.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.

**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-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 μg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

**Quality Control Assays**

**Exonuclease Assay:** Incubation of a 50 μl reaction containing 100 units of T4-BGT with 10 pmol of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA (10<sup>5</sup> cpm/μg) for 4 hours at 37°C released <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 nuclease 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 φX174 DNA for 4 hours at 37°C resulted in <10% conversion to RFI as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

## 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 Mfel.

**Heat Inactivation:** 65°C for 10 minutes

**Molecular Weight:** 40,666 kDa

### References:

1. Josse, J. and Kornberg, A. (1962). *J. Biol.Chem.*, 237, 1968–1976.
2. Tomaschewski, J. et al. (1985). *Nucleic Acids Res.* 13, 7551–7568
3. McNicol, L. A. et al. (1973) *J. Mol. Biol.* 15, 76, 285–301.
4. Szwagierczak, A. et al. (2010) *Nucleic Acids Res.* in press.

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