T4 Phage β-glucosyltransferase (T4-BGT)

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

M0357S 004160618061

M0357S

500 units	**10,000 U/ml**	**Lot: 0041606**
RECOMBINANT	**Store at –20°C**	**Exp: 6/18**

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-hydroxymethylcytosine (1,2).

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

Supplied in: 20 mM KPO_4, 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 [^{3}H]- or [^{14}C]-glucose into 5-hmC-containing DNA acceptor after incubation with [^{3}H]- or [^{14}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 [^{3}H] *E. coli* DNA (10^5 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 RFII 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 MfeI.

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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