**T4 Phage \(\beta\)-glucosyltransferase (T4-BGT)**

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 \([H]^-\) or \([\text{IC}]^-\)-glucose into 5-hmC-containing DNA acceptor after incubation with \([H]^-\) or \([\text{IC}]^-\)-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/rebsms.html).

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

**Reaction Conditions:** 1X NEBuffer 4 and 40 \(\mu\)M UDP-Glucose. Incubate at 37°C.

**Quality Control Assays**

**Exonuclease Assay:** Incubation of a 50 \(\mu\)l reaction containing 100 units of T4-BGT with 10 pmol of a mixture of single and double-stranded \([\text{IC}]^-\) E. coli DNA (10\(^5\) cpm/\(\mu\)g) for 4 hours at 37°C resulted in <0.1% of the total radioactivity.

**16-Hour Incubation:**
- A 50 \(\mu\)l reaction containing 1 \(\mu\)g of DNA and 100 units of T4-BGT for 16 hours at 37°C resulted in DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Endonuclease Assay:**
- Incubation of a 50 \(\mu\)l reaction containing 100 units of T4-BGT with 1 \(\mu\)g of \(\phi X174\) DNA for 4 hours at 37°C resulted in <10% conversion to RFII as determined by agarose gel electrophoresis.

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**M0357S**

500 units 10,000 U/ml Lot: 0041211

**RECOMBINANT Store at –20°C Exp: 11/14**

**Description:** T4 Phage \(\beta\)-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 (5-hmC) residues in double-diphosphoglucose (UDP-GlC) to the 5-hydroxy-methylcytosine (5-hmC) residues in double-diphosphoglucose (UDP-GlC).

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

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Enzyme Properties

Activity in NEBuffers:

<table>
<thead>
<tr>
<th>NEBuffer</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>100%</td>
</tr>
</tbody>
</table>

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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