β-Glucosyltransferase enzyme

Catalog No: 55012

Quantity: 500 Unit(s)
Concentration: 50,000 Unit(s)/mL

Reagents Supplied: β-Glucosyltransferase enzyme
10X β-Glucosyltransferase Reaction Buffer
50 mM UDP-Glucose
1 M DTT

Background: In mammalian genomes, DNA methylation usually occurs at the fifth carbon of cytosine residues. The recent discovery that the TET family of iron-dependent deoxygenases can convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) has raised questions about the functional relevance of 5-hmC in mammalian genomes. The **β-Glucosyltransferase enzyme** serves as a valuable tool to analyze 5-hmC by enabling modification of the 5-hydroxymethylcytosine residue with the addition of a glucose moiety. This modification can help with the differentiation of 5-mC from 5-hmC.

Enzyme Recognition Site: β-Glucosyltransferase will transfer the glucose moiety from the UDP-Glucose (uridine diphosphoglucose) to 5-hydroxymethylcytosine residues in double-stranded DNA.

Source: *E. coli* containing plasmid encoding the bacteriophage T4 β-Glucosyltransferase sequence.

Unit Definition: One unit is defined as the amount of enzyme required to fully protect 0.5 µg of 5-hmC DNA standard (Catalog No. 55008) in 1 hour at 37°C in a total reaction volume of 50 µl from cleavage by 4 units of GlaI restriction enzyme in an incubation of 16 hours at
30°C. A pilot titration of enzyme is recommended for protection of genomic DNA.

Reaction Conditions: DTT should be used at a 1 mM final concentration for each reaction. Prepare a fresh dilution of the provided 1 M DTT to 10 mM working stock in sterile water before use
(e.g. add 10 µl 1M DTT to 990 µl sterile water to make 1 ml of a 10 mM DTT working solution). Discard any unused 10 mM DTT solution after use.

The β-Glucosyltransferase enzyme is provided at a concentration of 50,000 units/ml. The enzyme should be diluted 1:10 in 1X Reaction Buffer prior to using. (e.g. Add 2 µl of β-Glucosyltransferase enzyme to 18 µl of 1X Reaction Buffer). We recommend using diluted enzyme within 2-weeks.

Example reaction:	
Sterile water	varies
10X Reaction Buffer	5 µl
10 mM DTT solution	5 µl
50 mM UDP-Glucose	2.5 µl
Sample DNA	varies
Diluted β-Glucosyltransferase enzyme	1 µl
Total Volume	50 µl

Incubate at 37°C for 1 hour.

Heat Inactivation: Heat inactivation is not recommended. The ß-Glucosyltransferase enzyme can be removed by purifying the reaction using Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002).

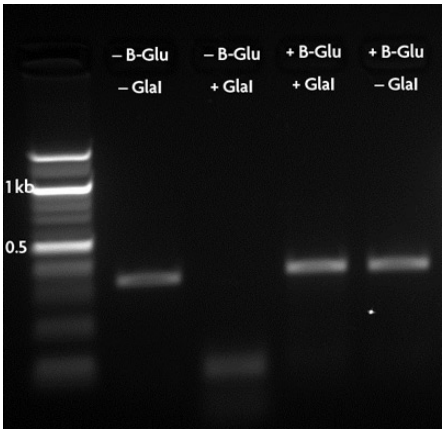

Figure 1: 0.5 µg of a 5-hmC Methylated DNA Standard (Catalog No. 55008) was incubated in the absence or presence of 1 unit β-Glucosyltransferase enzyme for 1 hour at 37°C. Following column purification, reactions were treated with 4 units of glucosyl-sensitive restriction enzyme GlaI and run on an 2.5% agarose gel.

Applications: β-Glucosyltransferase enzyme can be used to glucyosylate 5-hmC DNA. Glucosylated DNA can be used as a differentiator with glucosyl-sensitive restriction enzymes such as MspI, MspJI or GlaI (Figure 1). The use of a labeled [14C] UDP-Glucose donor would enable labeling of the 5-hmC residues.

General Notes: Reference: Szwagierczak, A. *et al.*, *Nucleic Acids Res* (2010) doi:10.1093/nar/gkq684.

Storage and Guarantee: The β-Glucosyltransferase enzyme is supplied in 50% glycerol and can be stored at -20°C. Make small aliquots and store at -80°C for prolonged storage. This product is guaranteed stable for 6 months from date of receipt when stored properly.