PRODUCT NAME Beta-Glucosyltransferase		
Full name : T4 Phage Beta (β)- glucosyltransferase		
Cat. No. : AF-112-0100	**Format :** 100 Reactions (100 µl)	**Concentration:** 1 U/µl

Product description:
The α- and β- glucosyltransferases of T-even bacteriophages catalyse the transfer of glucose from UDP-glucose (uridine diphospho-glucose) donor to genomic 5-hydroxymethylcytosine (hmC) bases [1]. It has been shown that β–glucosyltransferase can glucosylate hmC independently of DNA sequence and structural context. Actually, β- glucosyltransferase can glucosylate to completion various tested hmC-containing double-stranded DNA substrates both in vivo and in vitro, including the non-glucosylated T4 genome [2] and other complex genomes [4].

Recently it has been shown that β-glucosyltransferase can be used to discriminate between 5-mC and 5-hmC bases using glucosyl-sensitive restriction enzyme such as MspI, MspJI or GlaI. Moreover it has been successfully used for quantifying genomic 5-hydroxymethylcytosines bases using various labeling methods such as [14C] UDP-glucose radiolabeling [3] or keto-glucose followed by biotin linkage [4]. β-glucosyltransferase treatment is also a convenient method to check for the specificity during hMeDIP [5]. Recently, β-glucosyltransferase treatment was used as control in an anti-5-hmC dot blot assay since the anti-5hmC antibodies do not recognize glucosylated 5hmC [6].

Features:
- Isolated from a recombinant source
- Glucosylate to completion 5-hydroxymethylcytosine DNA bases
- Supplied with optimized 10X Reaction Buffer and UDP-Glucose donor
- Can be used to assess the global abundance of hmC in genomic DNA using glucosyl-sensitive restriction enzymes or labeling method combined with immunodetection [3,4, 5]
- Ideal to control hMeDIP experiments [5] and anti-5-hmC dot blot assays [6].

Source: Purified from an E. coli strain, which carries a bacteriophage T4 β-glucosyltransferase overexpressing plasmid.

Shipping conditions: Shipped at -20°C.

Storage conditions: Storage at -20°C is recommended. Since the 10x reaction buffer contains DTT, it is highly recommended to store it in small aliquots at -20°C. For long-term storage, store the enzyme, UDP-glucose donor and the 10X reaction buffer at -80°C. Avoid multiple freeze thawing. When stored properly, the enzyme is stable for at least 6 months from date of receipt.

Package content:
- 1 tube of β-glucosyltransferase (enough for 100 Reactions)
- 1 tube of 600µl of 10 X β-glucosyltransferase reaction buffer (enough for 100 x 50µl assays)
- 1 tube of 1 mM UDP-glucose donor (enough for 100 x 50 µl assays)

Unit definition:
1 unit is the amount of enzyme required to glucosylate 1µg of human genomic DNA in 20minutes at 25°C in 1x β-glucosyltransferase reaction buffer in a total volume of 50µl.

Reaction Conditions:
Thaw reagents completely and put them on ice. Mix all reagents well before use.

For a 50 µl glucosylation reaction :

10 x β-glucosyltransferase reaction buffer:	5 µl
DNA template:	2 µl
Glucosyltransferase (1 U/µl):	1 - 2 µl
1 mM UDP-glucose	5 µl
H_2O:	up to 50 µl

Incubate the reaction at 30°C for 1 hour. If needed the incubation time can be further extended up to 16 hours.

Important remark:

The amount of enzyme required to obtain full conversion of hmC into glucosylated hmC is dependent upon how much hmC is present in your sample DNA. Therefore some optimization might be required depending on the type or size of DNA used. Therefore it is highly recommended to test different incubation times and amount of enzymes.

Heat inactivation:
65°C for 10-15 minutes.

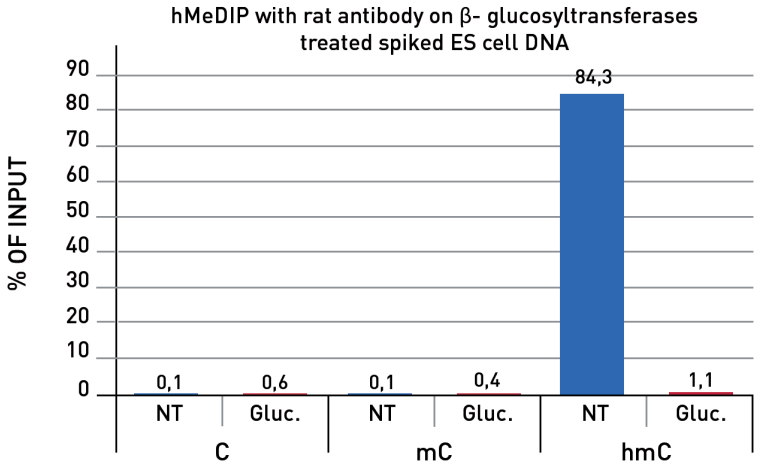

Figure 1
Mouse embryonic stem cells DNA was fragmented by sonication using the Diagenode Bioruptor® (size range: 100-500 bp). One µg of sheared mouse embryonic stem cells was mixed with the three controls (C, 5-mC and 5-hmC) from the hMeDIP kit. The DNA mixture was then treated enzymatically with (Gluc) or without (NT) β-glucosyltransferase as per the included reaction condition. After purification with the PrepEaseTM PCR Purification 96-well Plate kit (PN 78761, USB®), the reaction was recovered in 108 µl nuclease-free water and added to 12 µl of buffer H1 from the hMeDIP kit (Cat. No. AF-104-0016) The IP incubation mix was then heat denatured and hydroxymethylated DNA IP (hMeDIP) assays were performed using the Diagenode hMeDIP kit (Cat. No. AF-104-0016). This kit includes: the monoclonal rat antibody & Rat IgG (Cat. No. AF-105-0025). Finally qPCR using specific primer pairs for the unmethylated, methylated and hydroxymethylated control DNA sequences were performed. After glucosylation, the monoclonal rat antibody is not able to recognize hydroxymethylated epitopes.

Related products:

• PvuRts1I restriction enzyme	Cat. No : AF-113-0100
• 5-hmC, 5-mC & cytosine DNA standard pack (2µg)	Cat. No AF-101-0002
• hMeDIP kit x16 (for monoclonal mouse antibody)	Cat. No AF-110-0016
• hMeDIP kit x16 (for monoclonal rat antibody)	Cat. No AF-104-0016
• hMeDIP kit x16 (for polyclonal rabbit antibody)	Cat. No AF-111-0016
• 5-hmC monoclonal antibody (rat)	Cat. No : MAb-633HMC-100
• 5-hmC monoclonal antibody (mouse)	Cat. No : MAb-31HMC-100
• 5-hmC polyclonal antibody - crude (rabbit)	Cat. No : CS-HMC-100
• 5-hmC polyclonal antibody - purified (rabbit)	Cat. No : pAB-HMC-100

References:

1. Georgopoulos,C.P. and Revel,H.R., Studies with glucosyl transferase mutants of the T-even bacteriophages, Virology, 1971, 44, 271–285.
2. Kornberg,S.R., Zimmerman,S.B. and Kornberg,A., Glucosylation of deoxyribonucleic acid by enzymes from bacteriophage-infected Escherichia coli, J. Biol. Chem., 1961, 236,1487–1493.
3. Szwagierczak A., Bultmann S., Schmidt C.S., Spada F., Leonhardt H. Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA, Nucleic Acids Res., 2010, Aug 4. [Epub ahead of print] , doi:10.1093/nar/gkq684.
4. Song, C.X., Sun Y., Dai Q., Lu X.Y., Yu M., Yang C.G., He C. Detection of 5-Hydroxymethylcytosine in DNA by Transferring a Keto-Glucose by Using T4 Phage β-Glucosyltransferase. Chembiochem. 2011 Jun 7. doi: 10.1002/cbic.201100278.
5. Stroud H., Feng S., Morey Kinney S., Pradhan S., Jacobsen S.E. 5-hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. Genome Biol. 2011 Jun 20;12(6):R54.
6. Pastor WA, Pape UJ, Huang Y, Henderson HR, Lister R, Ko M, McLoughlin EM, Brudno Y, Mahapatra S, Kapranov P, Tahiliani M, Daley GQ, Liu XS, Ecker JR, Milos PM, Agarwal S, Rao A. Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. Nature 2011 May 19; 473(7347):394-7.

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