PROzyme®

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Agilent

β(1,4)-Galactosyltransferase

[β(1,4)-Galactosyltransferase 1: B4GalT1]

SPECIFICATIONS

Product Code: GKT-GA14
Activity: ≥12 U/mg
Storage: -20°C
Shipped on ice pack for next day delivery.
Formulation: 50 m*M* Tris HCl 100 m*M* NaCl (pH 8.4)

β(1,4)-Galactosyltransferase [β(1,4)-Galactosyltransferase 1, EC 2.4.1.38] is a truncated version (amino acids 45-398) of human GalT, the enzyme responsible for the synthesis of Galβ1-4GlcNAc. β(1,4)-Galactosyltransferase was cloned and expressed in HEK 293F cells.

β(1,4)-Galactosyltransferase (B4GalT1) transfers galactose from a donor substrate, UDP-galactose (UDP-Gal), to GlcNAcβ1-2Man units on glycoproteins and complex molecules.

Applications:

For *in vitro* galactosylation of glycoproteins such as monoclonal antibodies.

PRODUCT DESCRIPTION

Supplied Reagents:

WS0324 UDP-Gal
(3 X 10 mg; Uridine-5'-diphospho-galactose disodium salt)

WS0325 5x Reaction Buffer for GKT-GA14, 1 ml (50 m*M* $MnCl_2$, 500 m*M* MES, pH 6.5)

Molecular Weight: 39.5 kDa (by cDNA)

pH:

Recommended:	6.5
Range:	6.0 - 9.0

NOTE: A pH range of 6.0 to 9.0 may be used; at pHs below 6.5 enzyme activity may decrease; precipitation of $MnCl_2$ may occur at pHs above 7.0.

Stability: Store enzyme at -20°C. Avoid repeated freeze-thaw cycles as this decreases the efficacy of the enzyme. Dispense working aliquots (15 µg) after initial thaw if not utilizing entire quantity.

ASSAY

One unit of β(1,4)-Galactosyltransferase is defined as the amount of enzyme required to release one mmole of UDP from UDP-Gal (measured as βNAD at 340 nm) per minute, at a dilution of ~0.1-0.2 U/ml, pH 8.0 and 30°C.

SUGGESTIONS FOR USE

Before use, briefly centrifuge the vial to ensure that all material is at the base of the vial. Ensure that reagents, substrates and laboratory-ware are free from contaminants and proteases.

The amount of enzyme required for galactosylation and the amount of galactosylation will vary depending on the target molecule (IgG, Fc-fusion, *etc.*) or application.

The recommended incubation time is 2-24 hours. Longer incubation times do not show intrinsic galactosidase activity. A time course to determine the optimal conditions for different intended targets is recommended.

The suggested buffer conditions for galactosylation are 10 m*M* $MnCl_2$, 100 m*M* MES, pH 6.5 with 10 m*M* UDP-Gal as the donor substrate.

Suggested Procedure for Galactosylation

To prepare a 500 µg reaction using a standard IgG:

1) Prepare 1x Reaction Buffer.

   For example, add 100 µl of the supplied 5x Reaction Buffer to 400 µl of ultrapure water.

   The resulting 1x buffer contains 10 m*M* $MnCl_2$ with 100 m*M* MES, pH 6.5.

2) Prepare the target IgG to yield a final concentration of 10 mg/ml in 1x Reaction Buffer.

3) Dissolve one vial (10 mg) of UDP-Gal in 330 µl of 1x Reaction Buffer.

4) Combine 500 µg (50 µl) of target IgG with 606 µg (20 µl) of UDP-Gal and 15 µg (3 µl) of β(1,4)-Galactosyltransferase.

5) Adjust final volume to 100 µl using 1x Reaction Buffer.

6) Incubate at 37°C for 7.5 hours; longer incubation times do not adversely affect enzyme activity.

7) Stop the incubation by freezing at -15 to -25°C.

*NOTE: To avoid the potential for reaction-related artifacts affecting downstream analysis (*e.g., in vitro and in vivo *biological assays, PK/PD studies, mass spectrometric methods,* etc.*), the removal of excess reagents (*e.g., *by MWCO filtration, gel filtration, affinity chromatography,* etc.*) may be required.*

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