Recombinant Human
β-1,4-Galactosyltransferase 1/B4GalT1  
Catalog Number: 3609-GT

DESCRIPTION
Source
Mouse myeloma cell line, NS0-derived  
Gly44-Ser398, with an N-terminal 6-His tag  
Accession # NP_001488

N-terminal Sequence Analysis
His
Predicted Molecular Mass
40 kDa

SPECIFICATIONS
SDS-PAGE
45-55 kDa

Activity
Measured by its ability to transfer galactose from UDP-galactose to N-Acetyl-D-glucosamine.  
The specific activity is >2,000 pmol/min/μg, as measured under the described conditions.

Endotoxin Level
<1.0 EU per 1 μg of the protein by the LAL method.

Purity
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Formulation
Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol
Materials
- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM MgCl₂, 10 mM MnCl₂ (supplied in kit), pH 7.5
- Recombinant Human β-1,4-Galactosyltransferase 1/B4GalT1 (rhB4GalT1) (Catalog # 3609-GT)
- Donor Substrate: UDP-Galactose (Sigma, Catalog # U4500), 10 mM stock in deionized water
- Acceptor Substrate: N-Acetyl-D-glucosamine (GlcNAc) (EMD, Catalog # 1079), 1 M stock in deionized water
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Dilute UDP-Galactose to 0.5 mM in Assay Buffer.
2. Dilute Coupling Phosphatase 1 to 20 μg/mL in Assay Buffer.
3. Dilute GlcNAc to 50 mM in Assay Buffer.
4. Prepare Reaction Mixture by combining equal volumes of 0.5 mM UDP-GalNAc, 20 μg/mL Coupling Phosphatase 1, and 50 mM GlcNAc.
5. Dilute rhB4GalT1 to 0.75 μg/mL in Assay Buffer.
6. Dilute 1 mM Phosphate Standard by adding 40 μL of the 1 mM Phosphate Standard to 360 μL of Assay Buffer for a 100 μM stock. This is the first point of the standard curve.
7. Continue standard curve by performing six one-half serial dilutions of the 100 μM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5.0 nmol per well.
8. Load 50 μL of each dilution of the standard curve into a plate. Include a curve blank containing 50 μL of Assay Buffer.
9. Load 20 μL of the 0.75 μg/mL rhB4GalT1 into the plate. Include a Control containing 20 μL of Assay Buffer.
10. Start the reaction by adding 30 μL of Reaction Mixture to the wells, excluding the standard curve and curve blank.
11. Seal plate and incubate at room temperature for 15 minutes.
12. Add 30 μL of the Malachite Green Reagent A to all wells. Mix briefly.
13. Add 30 μL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
14. Read plate at 620 nm (absorbance) in endpoint mode.
15. Calculate specific activity:

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\text{Specific Activity (pmol/min/μg) = \frac{Phosphate released* (nmol) \times (1000 \text{ pmol/nmol})}{Incubation time (min) \times amount of enzyme (μg)}}
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*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions Per Well:
- rhB4GalT1: 0.015 μg
- Coupling Phosphatase 1: 0.2 μg
- UDP-Galactose: 5 nmol
- GlcNAc: 500 nmol

PREPARATION AND STORAGE
Shipping
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.
β4GalT1 is one of seven β1,4 galactosyltransferases that transfer galactose in a β1,4 linkage to acceptor sugars including GlcNAc, and Glc, and Xyl. By sequence similarity, the β4GalTs form four groups: β4GalT1 and β4GalT2, β4GalT3 and β4GalT4, β4GalT5 and β4GalT6, and β4GalT7 (1). β4GalT1 is unique among the seven enzymes because it can be expressed either as membrane associated form or secreted form (2). The secreted form is restricted to lactating mammary tissues where the enzyme forms a heterodimer with α-lactalbumin to catalyze the synthesis of lactose (3). The membrane form can reside either in the Golgi apparatus, where it adds galactose to N-acetylglucosamine residues, or on cell surface, where it functions as a recognition molecule during a variety of cell to cell and cell to matrix interactions, by binding to specific oligosaccharide ligands on opposing cells or in the extracellular matrix (4). The two enzymatic forms result from alternate transcription initiation sites and post-translational processing (5). Defects in β4GalT1 are the cause of congenital disorder of glycosylation type 2D (CDG2D) (6). The activity of this enzyme has been measured with a phosphatase-coupled method (7).

References: