Recombinant Human β-1,4-Galactosyltransferase 2/B4GalT2

Catalog Number: 7530-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ser43-Gly372, with C-terminal 6-His tag Accession # O60909
N-terminal Sequence Analysis	Ser43
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	44-55 kDa, reducing conditions
Activity	Measured by its ability to transfer galactose from UDP-galactose to glucose. The specific activity is >80 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
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, 10 mM $MnCl_2$, 10 mM $CaCl_2$, 65 mM NaCl, pH 7.5
- Recombinant Human β-1,4-Galactosyltransferase 2/B4GalT2 (rhβ4GalT2) (Catalog # 7530-GT)
- UDP-Galactose (Sigma, Catalog # U4500), 10 mM stock in deionized water
- D-(+)-Glucose (Sigma, Catalog # G5767), 2 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 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.
2. 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.
3. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
4. Dilute rhβ4GalT2 to 16 µg/mL.
5. Prepare a Reaction Mixture composed of 0.4 M Glucose, 1 mM UDP-Galactose, and 4 µg/mL Coupling Phosphatase 1 in Assay Buffer.
6. Load 25 µL of the 16 µg/mL rhβ4GalT2 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Start the reaction by adding 25 µL of Reaction Mixture to the wells, excluding the standard curve and curve blank.
8. Cover the plate with a plate sealer and incubate at room temperature for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Well:

- rhβ4GalT2: 0.4 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-Galactose: 0.5 mM
- Glucose: 200 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

There are seven β-1,4-galactosyltransferases that transfer galactose in a β-1,4 linkage to acceptor sugars including GlcNAc, Glc, and Xyl. By sequence similarity, the β4GalTs form four groups: β4GalT1 and β4GalT2, β4GalT3 and β4GalT4, β4GalT5 and β4GalT6, and β4GalT7 (1). All of these enzymes are expressed as type II membrane proteins in Golgi apparatus except β4GalT1, which can be expressed as a secreted form in lactating mammary tissues due to an alternative transcription initiation site (2, 3). β4GalT2 is responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins as well as the carbohydrate moieties of glycolipids (4). It can also produce lactose. Its substrate specificity is affected by α-lactalbumin, but it is not expressed in lactating mammary tissue (5). Recently, β4GalT2 has been shown to form a complex with GlcAT-P to synthesize HNK-1 carbohydrate in the nervous system (6). The activity of this enzyme has been measured with a phosphatase-coupled method (7).

References:

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