Recombinant Porcine α3GalT/GGTA1 His-tag

Catalog Number: 11261-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived porcine alpha3GalT/GGTA1 protein Glu23-Ile371, with a C-terminal 6-His tag Accession # P50127-1
N-terminal Sequence Analysis	Glu23
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	39-48 kDa, under reducing conditions.
Activity	Measured by its ability to transfer galactose from UDP-galactose to α-Lactose. The specific activity is >400 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	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Glycosyltransferase Activity Kit (Catalog # EA001)
- Phosphate Buffer 1: 10X solution of 250 mM Tris, 100 mM $CaCl_2$, pH 7.5 (provided in kit)
- $MnCl_2$: 100 mM $MnCl_2$ in deionized water (provided in kit)
- Recombinant Porcine α3GalT/GGTA1 His-tag (rpGGTA-1) (Catalog # 11261-GT)
- UDP-Galactose, 10 mM stock in deionized water
- α-Lactose, 0.3 M stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader

Assay

1. Prepare 1X Assay Buffer (25 mM Tris, 10 mM $CaCl_2$, 10 mM $MnCl_2$, pH 7.5) containing 10 mM $MnCl_2$ by combining equal volumes of Phosphate Buffer 1 and 100 mM $MnCl_2$ and diluting 5-fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Dilute Coupling Phosphatase 1 (provided in kit) to 10 µg/mL in 1X Assay Buffer.
5. Prepare a Reaction Mixture containing 0.8 mM UDP-Galactose, 40 mM α-Lactose, and 4 µg/mL Coupling Phosphatase 1 in 1X Assay Buffer.
6. Dilute rpGGTA-1 to 5 µg/mL in 1X Assay Buffer.
7. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
8. Load 25 µL of 5 µg/mL rpGGTA-1 into empty wells of the same plate as the curve. Include a Control containing 25 µL of 1X Assay Buffer.
9. Start the reaction by adding 25 µL of Reaction Mixture to all wells, excluding standard curve.
10. Seal and incubate plate at 37 °C for 20 minutes.
11. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
12. Add 100 µL of deionized water 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:

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

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

Final Assay Conditions

Per Reaction:

- rpGGTA-1 0.125 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-Galactose 0.4 mM
- α-Lactose: 20 mM

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.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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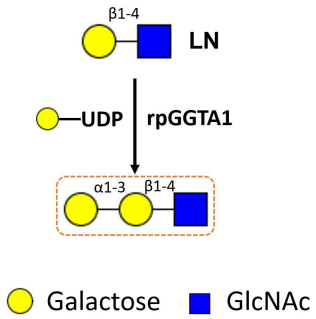
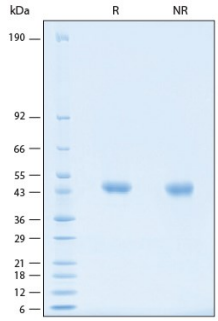
DATA

Enzyme Activity



Recombinant Porcine alpha3GalT/GGTA1 His-tag Enzyme Activity Diagram. Recombinant Porcine alpha3GalT/GGTA1 His-tag Protein (Catalog # 11261-GT) introduces an α1-3 linked galactose residue to terminal lactosamine (LN) structure and generates α-Gal epitope (boxed).α-Gal is ubiquitously presented in non-primate mammals but absent in humans and is responsible for hyperacute rejection of organs transplanted from α-gal donors. The enzyme shows reduced activity on lactose, which is the basis for the current enzyme activity assay.

SDS-PAGE



Recombinant Porcine alpha3GalT/GGTA1 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Porcine alpha3GalT/GGTA1 His-tag Protein (Catalog # 11261-GT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 39-48 kDa.

BACKGROUND

GGTA1, also designated as α3GalT, is an α1,3 galactosyltransferase that catalyzes the transfer of galactose from UDPαDgalactose to various glycoconjugates to form nonreducing terminal α1,3linked galactosyl moieties, or α-Gal epitope (1). This enzyme is expressed in many mammalian species but is absent from humans, apes, and Old World monkeys due to the mutational inactivation of the gene (2). Therefore, humans do not have α-Gal on glycoconjugates, but produce large amounts of antibody to this epitope, resulting from exposure to α-Gal containing antigens (3). GGTA1 is of great medical interest because the immune responses may be enhanced when selected targets are decorated with α-Gal structures (4). Additionally, the presence of anti-α-Gal antibodies is a barrier to xenotransplantation of organs. Recombinant pig GGTA1 may provide easy access to a wide spectrum of α-Gal epitopes and their derivatives to support studies on xenotransplantation and other pharmaceutical research (5, 6). Structurally and mechanistically GGTA1 is a model for several homologous glycosyltransferases that differ in donor and acceptor substrate specificity, including the histo blood group A and B glycosyltransferases, Forssman glycolipid synthase, and isogloboside 3 synthase (7).

References:

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449