Recombinant Human GALT His-tag

Catalog Number: 11776-GT

DESCRIPTION

Source	*E. coli*-derived human GALT protein Ser2-Ala379, with an N-terminal Met and 6-His tag Accession # P07902.3
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	40-45 kDa, under reducing conditions.
Activity	Measured by its ability to hydrolyze UDP-Glucose. The specific activity is >1400 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $MnCl_2$, pH 7.0
- Enzyme: Recombinant Human GALT His-tag (rhGALT) (Catalog # 11776-GT)
- NADP+, 50 mM stock in deionized water
- Substrate: Galactose 1-phosphate, 50 mM stock in deionized water
- UDP-Glucose, 10 mM stock in 25% Ethanol/75% deionized water
- Coupling Enzyme: Recombinant Human G6PD His-tag (rhG6PD) (Catalog # 10096-DH)
- Coupling Enzyme: Recombinant Human PGM-1 N-His (rhPGM-1) (Catalog # 11681-P1)
- Clear 96-well Plate
- Plate Reader with Absorbance Read Capability

Assay

1. Create a master mix containing 0.25 mM NADP+, 1 mM Galactose 1-phosphate, 0.25 mM UDP-Glucose, 20 µg/mL rhG6PD, and 40 µg/mL rhPGM-1 in Assay Buffer.
2. Dilute rhGALT to 2.5 µg/mL in Assay Buffer.
3. Load in a plate 50 µL of 2.5 µg/mL rhGALT, and start the reaction by adding 50 µL of master mix. Include a Control containing 50 µL of master mix and 50 µL of Assay Buffer.
4. Read plate at 340 nm (absorbance) in kinetic mode for five minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}^{*} \text{ (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} \text{ } (M^{-1}cm^{-1}) \text{ x path corr.}^{***} \text{ (cm) x amount of enzyme (µg)}}$$

*Adjusted for Control

**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: The output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhGALT: 0.125 µg
- NADP+: 0.125 mM
- Galactose 1-phosphate: 0.5 mM
- UDP-Glucose: 0.125 mM
- rhG6PD: 1 µg
- rhPGM-1: 2 µg

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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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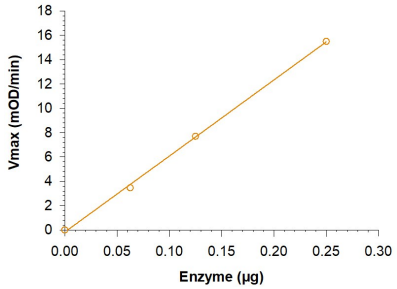
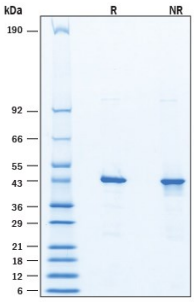
Enzyme Activity



Recombinant Human GALT His-tag Enzyme Activity. Recombinant Human GALT His-tag (Catalog # 11776-GT) is measured by its ability to hydrolyze UDP-Glucose.

SDS-PAGE



Recombinant Human GALT His-tag SDS-PAGE. 2 µg/lane of Recombinant Human GALT His-tag (Catalog # 11776-GT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 40-45 kDa, under reducing conditions.

BACKGROUND

Galactose-1-phosphate uridylyltransferase (GALT), also known as UDP-glucose-hexose-1-phosphate uridylyltransferase, is a ubiquitously expressed cytoplasmic protein that belongs to the histidine triad superfamily (1-3). GALT is a key enzyme in the Leloir pathway involved in the conversion of the essential monosaccharide galactose into glucose (1, 3). In addition, GALT inter-converts uridine diphosphate (UDP) hexoses used in the formation of glycogen and glycoconjugates and is involved in the metabolism of UDP-N-acetyl-hexose-amines, which are substrates of glycosyltransferases and highly important structural elements of glycosaminoglycans (3, 4). GALT is a homodimer where each ~43 kDa monomer contains a structurally important zinc-binding site, a glucose-1-phosphate binding site, and a covalent uridylylated histidine in a conserved active site present at the interface between the subunits. Uridylylation induces a conformational change to reduce flexibility and therefore both uridylylation and zinc binding influence the stability and aggregation tendency of hGALT. Autosomal recessive inherited variants of GALT that disrupt zinc-binding or reduce the ability to form the uridylylated intermediate cause classic galactosemia (or type I galactosemia) (3). Hallmarks of classic galactosemia include elevated levels of the metabolite galactose 1-phopshate (5), reduced levels of UDP-hexoses (6) and disturbed glycosylation (3, 7-9) that are caused by mutations in GALT and lead to misfolding and dysfunctional enzymatic activity through effects on expression, solubility, stability and aggregation tendency (1, 3, 10-12). GALT is being explored as a target for gene therapy, and both the GALT dimer interface active sites and the divalent metal binding sites are targets for small molecule design and screening for galactosemia treatments (3, 12, 13). GALT activity is currently used for newborn screening and diagnosis of galactosemia (14). In addition, GALT's role in sugar metabolism makes it a potential tool for use in broader synthetic biology applications (3).

References:

1. Elsas, L.J. and K. Lai. (1998) Genet. Med. **1**:40.
2. Brenner, C. (2002) Biochemistry **41**:9003.
3. McCorvie, T.J. *et al* (2016) Hu. Mol. Genet. **25**:2234.
4. Weckbecker, G. and D.O. Keppler. (1982) Eur. J. Biochem. **128**:163.
5. Donnell, G. *et al* (1963) Pediatrics **31**:802.
6. Lai, K. *et al* (2003) Glycobiology **13**:285.
7. Staubach, S. *et al.* (2012) J. Proteome Res. **11**: 906.
8. Coss, K.P. *et al.* (2014) J. Proteome Res. **13**: 385.
9. Maratha, A. *et al.* (2016) Eur. J. Hum. Genet. **24**:976.
10. Tang, M. *et al.* (2012) Hum. Mutat. **33**: 1107.
11. Coelho, A.I. *et al.* (2014) Mol. Genet. Genomic Med. **2**:484.
12. Banford, S. *et al.* (2021) J. Pers. Med. **11**:106.
13. Delnoy, B. *et al.* (2021) J. Pers. Med. **11**:75.
14. Pasquali, M. *et al.* (2018) Genet. Med. **20**:3.

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