

PROTOCOL

We recommend the use of Low Protein Binding Polypropylene Tubes (0.5-1.5 mL)

1. Add to a polypropylene tube:
 - 5 μ l HEPES Buffer at 500 mM, pH 7.5
 - 0.5 μ L 0.5 M $MnCl_2$
 - Acceptor molecule to a final concentration between 0.5 mM and 5 mM.
 - UDP-galactose to a final concentration at least 1.5 times higher than acceptor.
 - 10 μ L of B3GalT (add enzyme always in the last place)
2. Mix by gently tapping the tube
3. Incubate at 37°C for 1 hour.
4. Analyze results by HPLC, UHPLC or MALDI-TOF

B3GalT

β eta-(1,3)-GalactosylTransferase

4 mU

+34 943 38 22 74

<https://aspariaglycomics.com>

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INSTRUCTIONS

Instruction for B3GalT (4 mU).

Upon arrival store enzyme at -80 °C.

Introduction

B3GalT is a recombinant N-acetyl-beta-D-glucosaminide beta-(1,3)-galactosyltransferase. The enzyme transfers galactose from UDP-galactose to the N-acetylglucosamine termini of acceptor molecules such as GalNAc, Lacto-N-triose, Globotetrose, GalNAc β 1,3Gal β 1,4Glc Isoglobotetrose or GlcNAc. B3GalT has been used to produce the Human Milk Oligosaccharide (HMO) lacto-N-tetraose (LNT) and N-Acetyllactosamine (LacNAc) Type 1 Oligomers.

The enzyme is provided in solution containing Tris buffer, NaCl and 10% glycerol.

Activity: The activity per μ L is 40 μ Units/ μ L or 248 pmol/min/ μ L.

Milliunit Definition: One milliunit will transfer 1.0 nanomole of galactose from UDP-galactose to 4-Nitrophenyl N-acetyl- β -D-glucosaminide per min at pH 7.5 at 37 °C.

Enzyme Thawing

1. Thaw the enzyme quickly by warming in the hands.
2. Mix gently by tapping the tube.
3. Place immediately on ice and keep enzyme always on ice

Storage

For short term storage (days) store at 4°C.

For long term storage (months) store at - 80°C.

Avoid repeated freeze-thaw cycles, which decrease enzyme stability.