



## CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO®  $\beta$  (1-2,3,4,6) HEXOSAMINIDASE SEQUENCING-GRADE (Jack Bean)

PRODUCT CODE: GKX-5023

LOT NUMBER: 183 010a

FORMULATION: A sterile-filtered solution in 20 mM sodium citrate phosphate (pH 6.0)

STORAGE: 2-8°C

PACK SIZE: 15 Units

FILL VOLUME: 50  $\mu$ l per vial

DIRECTIONS FOR USE: See back of page for applications suggestions

EXPIRATION: November 2018

### QUALITY CONTROL

1. Activity <sup>1</sup> :	Passed	(Specification: $\geq 300$ U/ml)
2. Specific activity:	Passed	(Specification: $\geq 40$ U/mg)
3. Protease assay <sup>2</sup> :	Passed	(Specification: "Not Detectable")
4. Contaminants <sup>3</sup> :	Passed	(Specification: $\leq 0.01\%$ )

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Authorized Signature

1. One unit of  $\beta$ -N-Acetylhexosaminidase is defined as the amount of enzyme required to catalyze the release of 1  $\mu$ mole of p-nitrophenol from pNP-N-acetyl- $\beta$ -D-glucosaminide per minute at pH 5.0 and 37°C.
2. No protease activity was detectable after incubation of the enzyme with 0.2 mg resorufin-labeled casein for ~18 hours at 37°C based on Schickaneder E, Hösel W, von der Eltz H, Geuß U. Casein-resorufin, a new substrate for a highly sensitive protease assay. Fresenius Z. Anal Chem. 1988 330:360.
3. The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP-glycosides:  $\alpha$ -mannosidase,  $\beta$ -mannosidase  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -fucosidase,  $\beta$ -fucosidase,  $\alpha$ -N-acetylgalactosaminidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase and  $\beta$ -xylosidase. The product was tested for contaminating sialidase by extended incubation with MU-NANA.

#### DIRECTIONS FOR USE:

Before use, briefly centrifuge the vial to ensure that all material is at the base of the vial. For the digestion of isolated glycans incubations should be performed at pH 4-5 at an enzyme concentration of 10-20 U/ml. Higher enzyme concentrations (50 U/ml) may be necessary to completely remove bisecting  $\beta(1-4)$ -linked GlcNAc residues in complex N-glycans, which are generally more resistant to enzymatic hydrolysis. As a guideline, digestions with isolated glycans should be performed for 16-24 hours at 37°C at a substrate concentration of approximately 20  $\mu M$ .

#### APPLICATIONS:

The enzyme exhibits a broad specificity, cleaving non-reducing terminal  $\beta(1-2,3,4$  and 6)-linked N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) residues. It has proved very useful in the study of isolated glycans, glycolipids and glycoproteins, especially in combination with  $\beta$ -N-Acetylhexosaminidase from *S. pneumoniae* (GK80050).

The enzyme shows different pH optima for the hydrolysis of pNP-N-acetyl- $\beta$ -D-glucosaminide (pH 5.0-6.0) and pNP-N-acetyl- $\beta$ -D-galactosaminide (pH 3.5-4.0). At pH 4.5 the relative rates of hydrolysis of each substrate are virtually equal.