

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® β (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 μ l per vial
DIRECTIONS FOR USE: See back of page for applications suggestions
EXPIRATION: May 2020 (extended from prior exp. date based on re-assay)
RE-ASSAY DATE: May 2019

QUALITY CONTROL

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

Authorized Signature

1. One unit of β -N-Acetylhexosaminidase is defined as the amount of enzyme required to catalyze the release of 1 μ mole of p-nitrophenol from pNP-N-acetyl- β -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: α -mannosidase, β -mannosidase α -galactosidase, β -galactosidase, α -fucosidase, β -fucosidase, α -N-acetylgalactosaminidase, α -glucosidase, β -glucosidase and β -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 μM .

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 β -N-Acetylhexosaminidase from *S. pneumoniae* (GK80050).

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