



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® ENDO F2 (recombinant from *Elizabethkingia meningoseptica*, expressed in *E. coli*)

NOTE: The source organism was previously known as *Chryseobacterium [Flavobacterium] meningosepticum*

PRODUCT CODE: GKE-5008

LOT NUMBER: DG68 014a

FORMULATION: A sterile-filtered solution in 10 mM sodium acetate, 25 mM NaCl, 0.02% sodium azide (pH 4.5)

STORAGE: 2-8°C

PACK SIZE: 6 mU

FILL VOLUME: 60 µl per vial

SUGGESTIONS FOR USE: Conditions for use vary depending on the application. Suggested protocol: To 100 µg of glycoprotein, add de-ionized water to a total of 82 µl. Add 17 µl of WS0161 5x Reaction Buffer for GKE-5008 and 1 µl of Endo F2 to the reaction vial. Incubate for 3 hours at 37°C. Monitor cleavage by SDS-PAGE (see example on page 2, figure 1).

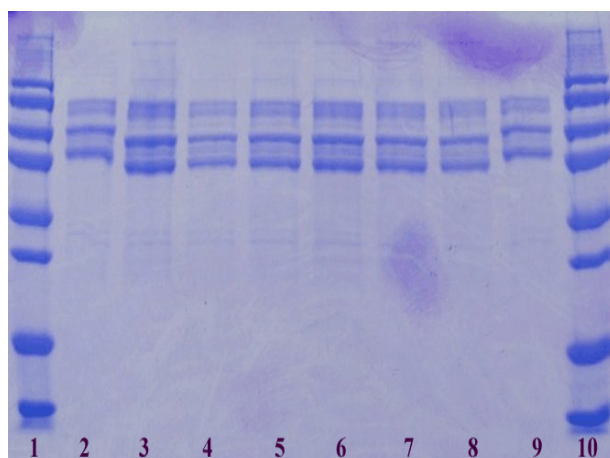
QUALITY CONTROL

1.	Activity ¹ :	Passed	(Specification: ≥100 mU/ml)
2.	Protease assay ² :	Passed	(Specification: "Not Detectable")
3.	Contaminants ³ :	Passed	(Specification: ≤0.1%)

Authorized Signature

- One milliunit of Endo F2 is defined as the amount of enzyme required to catalyze the release of the glycan from 1 nanomole of dansylated porcine fibrinogen glycopeptide substrate, DnsVGEN(CHO)R, per minute at pH 4.5 and 37°C (under initial rate conditions). **NOTE: The activity of this material is equivalent to material formerly sold by Glyko® and Oxford GlycoSciences as E-5008.**
- 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.
- The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP-glycosides: α-fucosidase, β-fucosidase, α-mannosidase, β-mannosidase, α-galactosidase, β-galactosidase, β-N-acetylhexosaminidase, α-N-acetylgalactosaminidase, α-glucosidase, β-glucosidase and β-xylosidase. The absence of contaminating sialidase was confirmed by extended incubation with MU-NANA.

Example Deglycosylation of 0.5 mg Fibrinogen with GKE-5008 Endo F2¹



- Lane 1: Molecular Weight Markers
Lane 2: Blank (no enzyme)
Lane 3: Reaction 1 - 1 μ l GKE-5008 Endo F2, 5 hour incubation
Lane 4: Reaction 2 - 5 μ l GKE-5008 Endo F2, 5 hour incubation
Lane 5: Reaction 3 - 8 μ l GKE-5008 Endo F2, 5 hour incubation
Lane 6: Reaction 4 - 1 μ l GKE-5008 Endo F2, overnight incubation
Lane 7: Reaction 5 - 5 μ l GKE-5008 Endo F2, overnight incubation
Lane 8: Reaction 6 - 8 μ l GKE-5008 Endo F2, overnight incubation
Lane 9: Blank (no enzyme)
Lane 10: Molecular Weight Markers

PROTOCOL NOTES:

Each reaction contained 0.5 mg of porcine fibrinogen in a total volume of 100 μ l of 200 mM sodium acetate, 2 mM NaCl, 0.0016% sodium azide (pH 4.5). The reactions were incubated for 5 hours or overnight at 37°C, then were terminated using a dry ice acetone bath. 5 μ l of each reaction mixture was loaded on a 12% SDS-polyacrylamide gel.

¹Not lot-specific data.