O-Glycosidase and Neuraminidase Bundle

Source: O-Glycosidase is cloned from Enterococcus faecalis and expressed in E. coli (1). Neuraminidase is cloned from Clostridium perfringens (1) and overexpressed in E. coli at NEB (2).

Reagents Supplied with Enzymes:
10X Glycoprotein Denaturing Buffer, 10X GlycoBuffer 2, 10% NP-40

Reaction Conditions:
Typical reaction conditions are as follows:
1. Combine 10–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H2O (if necessary to make a 10 µl total reaction volume).
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 µl by adding 2 µl 10X GlycoBuffer 2, 2 µl 10% NP-40, 2 µl Neuraminidase, H2O and 1–5 µl O-Glycosidase.
4. Incubate reaction at 37°C for 1–4 hours. Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Unit Definition of O-Glycosidase: One unit is defined as the amount of enzyme required to remove equivalent molar amounts of O-linked disaccharides and N-linked oligosaccharides, respectively.

Non-denaturing Unit Definition Assay: Two fold serial dilutions of O-Glycosidase are added to a reaction mixture of 5 µg of Neuraminidase digested fetuin with 1X GlycoBuffer 2. The reaction is then incubated at 37°C for 1 hour. O-linked disaccharide carbohydrates are determined by Morgan and Elson Assay (4). Note: Under denaturing conditions the enzyme activity is increased two-fold. This observation is substrate dependent.

Unit Definition of Neuraminidase: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5AcXX2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

Specific Activity of O-Glycosidase:
~50,000,000 units/mg.

Molecular Weight of O-Glycosidase: 147,000 daltons.

Specific Activity of Neuraminidase:
~225,000 units/mg.

Molecular Weight of Neuraminidase: 43,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls
Glycosidase Assays: 200,000 units of O-Glycosidase and 500 units of Neuraminidase were incubated with 0.1 mM of fluorecently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

No other glycosidase activities were detected (ND) with the following substrates:

- N-Acetylgalactosaminidase: GalNAcβ1-4Galβ1-4Glc-AMC ND
- N-Acetylgalactosaminidase: GalNAcα1-3(Fucxx1-2)Galβ1-4Glc-AMC ND
- Fucosidase: Galβ1-4(Fucxx1-2)GalNAcβ1-3Galβ1-4Glc-AMC ND
- Galactosidase: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- Galα1-4GalNAcβ1-3Galβ1-4Glc-AMC ND
- Galα1-3Galβ1-4Gal-AMC ND
- Galα1-3Galβ1-4Glc-AMC ND
- Manα1-3Manβ1-4GlcNAc-AMC ND
- Manα1-6Manα1-6(Manα1-3)Man-AMC ND
- Glcβ1-4Glcβ1-4Glc-AMC ND
- Glcα1-6Glcxx1-2Fru-AMC ND
- Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase: Manβ1-4Manβ1-4Man-AMC ND
Endo F1, F4, H: Dansylated invertease high mannose. ND
Endo F1, F4: Dansylated fibrinogen biantennary. ND
PNGase F: Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 1,400,000 units of O-Glycosidase and 500 units of Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Note: Since O-Glycosidase is inhibited by SDS, it is essential to have NP-40 in the reaction mixture. It is not known why this non-ionic detergent counteracts the SDS inhibition at the present time. Double digest with Endo H must have NP-40 present (NP-40 does not inhibit Endo H).

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

Recommended storage temperature is ~20°C.

References: