3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Native Protein Deglycosylation Kit

Catalog Number **NDEGLY**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The NDEGLY Kit is intended for the deglycoslylation of N-linked oligosaccharides from glycoproteins under native conditions. Particular residues, due to their location in the native protein structure, are often resistant to the traditional deglycosylation methods using PNGase F and cannot be removed unless the protein is denatured.

Endoglycosidases F1, F2, and F3 are less sensitive to protein conformation than PNGase F and are more suitable for deglycosylation of native proteins. The linkage specificities of Endoglycosidases F1, F2, and F3 suggest a general strategy for deglycosylation of proteins that may remove all classes of N-linked oligosaccharides without denaturing the protein (see Figure 1). All complex oligosaccharides can be reduced to the trimannosylchitobiose core by treatment of the glycoproteins with neuraminidase, β-galactosidase, and N-acetylglucosaminidase, available as part of the EDEGLY Kit (see Figure 2). Fucosidases may be required in some situations. The remaining trimannosyl-chitobiose core structures can be removed with Endoglycosidase F3. Biantennary and triantennary structures can be immediately removed by Endo-glycosidases F2 and F3, respectively. Oligomannose and hybrid structures can be removed by Endo-glycosidase F1.

Reagents

Endoglycosidase F1 (Catalog Number E9762)	0.3 unit
Endoglycosidase F2 (Catalog Number E0639)	0.1 unit
Endoglycosidase F3 (Catalog Number E2264)	0.1 unit
Endoglycosidase F1 Reaction Buffer (Catalog Number R9025)	200 µl
Endoglycosidase F2 and 3 Reaction Buffer (Catalog Number R9150)	200 µl

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The NDEGLY Kit ships on wet ice and storage at 2–8 °C is recommended. This kit may be used for at least 1 year when stored as indicated. Several days exposure to ambient temperatures will not reduce the activity of the enzymes in this kit.

Endoglycosidase F1
from *Elizabethkingia miricola*
recombinant, expressed in *E. coli*
Catalog Number **E9762**

EC 3.2.1.96
Synonyms: Endo-β-N-acetylglucosaminidase F1, Endo F1
Elizabethkingia miricola was formerly known as *Elizabethkingia, Chryseobacterium,* or *Flavobacterium meningosepticum*.

The type of carbohydrate structure, oligomannose, hybrid or complex, and the state of α(1→6) core fucosylation have profound effects on the cleavage by the endoglycosidases. Endoglycosidase F1 cleaves asparagine-linked or free oligomannose and hybrid, but not complex, oligosaccharides. Core fucosylation of hybrid structures reduces the rate of cleavage by Endo F1 more than 50-fold. The core fucosylated hybrids are usually only found on glycoproteins from swainsonine-treated cells. Endo F1 will cleave sulfated high-mannose oligosaccharides; whereas, Endo H will not. Endo F1 cleaves between the two N-acetyl-glucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact (see Figure 3).

The enzyme has a molecular mass of ~32 kDa.

The enzyme is provided as a solution in 20 mM Tris-HCl, pH 7.5.

Deglycosylation Procedure

1. Add up to 200 µg of glycoprotein to a micro-centrifuge tube. Adjust the final volume to 37.5 µl with deionized water.
2. Add 10 µl of 5× Reaction Buffer (Catalog Number R9025)
3. Add 2.0 µl of Endo F1 solution to the reaction. Incubate 1 hour at 37 °C.
4. Monitor cleavage by SDS-PAGE.

Endoglycosidase F2
from *Elizabethkingia miricola*
recombinant, expressed in *E. coli*
Catalog Number **E0639**

EC 3.2.1.96
Synonyms: Endo-β-N-acetylglucosaminidase F2, Endo F2
Elizabethkingia miricola was formerly known as *Elizabethkingia, Chryseobacterium,* or *Flavobacterium meningosepticum*.

Endo F2 and Endo F3 are unique in their ability to cleave complex structures. Endo F2 cleaves asparagine-linked or free oligomannose, and biantennary complex oligosaccharides. Oligomannose structures are cleaved at a 20-fold reduced rate. Fucosylation has little effect on Endo F2 cleavage of biantennary structures. It will not cleave hybrid structures. It cleaves between the two N-acetyl-glucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. Endo F2 is less sensitive to protein conformation than PNGase F and is more suitable for deglycosylation of native proteins.

The enzyme has a molecular mass of ~32 kDa.

The enzyme is provided as a solution in 10 mM sodium acetate and 25 mM sodium chloride, pH 4.5.

Deglycosylation Procedure

1. Add up to 200 µg of glycoprotein to a micro-centrifuge tube. Adjust the final volume to 37.5 µl with deionized water.
2. Add 10 µl of 5× Reaction Buffer (Catalog Number R9150)
3. Add 2.0 µl of Endo F2 solution to the reaction. Incubate 1 hour at 37 °C.
4. Monitor cleavage by SDS-PAGE.

Endoglycosidase F3
from *Elizabethkingia miricola*
recombinant, expressed in *E. coli*
Catalog Number **E2264**

EC 3.2.1.96
Synonyms: Endo-β-N-acetylglucosaminidase F3, Endo F3
Elizabethkingia miricola was formerly known as *Elizabethkingia, Chryseobacterium,* or *Flavobacterium meningosepticum*.

Endo F3 is unique in that its cleavage is sensitive to the state of peptide linkage of the oligosaccharide, as well as the state of core fucosylation. Endoglycosidase F3 cleaves asparagine-linked biantennary and triantennary complex oligosaccharides. It will cleave non-fucosylated biantennary and triantennary structures at a slow rate, but only if peptide-linked. Core fucosylated biantennary structures are efficient substrates for Endo F3, even as free oligosaccharides. Endo F3 will also cleave fucosylated trimannosyl core structures on free and protein-linked oligosaccharides. Core fucosylation of biantennary structures increases activity up to 400-fold. There is no activity on oligomannose and hybrid molecules. It will only cleave non-fucosylated trimannosyl core structures when attached to a protein, not as free oligosaccharides. This activity was previously attributed only to Endoglycosidase D from *Diplococcus (Streptococcus) pneumonia* (see Figure 4). The effect of fucosylation on triantennary structures has not yet been investigated. Endo F3 cleaves between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact. When used in conjunction with the EDEGLY Kit, all complex oligosaccharides can be reduced to the trimannosylchitobiose core with subsequent removal with Endo F3.

Endo F3 is less sensitive to protein conformation than PNGase F and is, therefore, more suitable for deglycosylation of native proteins.

The use of detergents for Endo F3 digestions should be avoided.

The enzyme has a molecular mass of ~30 kDa.

The enzyme is provided as a solution in 20 mM Tris-HCl, pH 7.5.

Deglycosylation Procedure

1. Add up to 200 µg of glycoprotein to a micro-centrifuge tube. Adjust the final volume to 37.5 µl with deionized water.
2. Add 10 µl of 5× Reaction Buffer (Catalog Number R9150)
3. Add 2.0 µl of Endo F3 solution to the reaction. Incubate 1 hour at 37°C.
   Note: When cleaving non-core fucosylated biantennary and triantennary N-linked oligo-saccharides, increase incubation time to 4 days. Many glycoproteins are contaminated with proteases. We advise adding suitable protease inhibitors if long duration incubations are anticipated.
4. Monitor cleavage by SDS-PAGE.

Figure 1.
Specificities of Endoglycosidases F1, F2, and F3

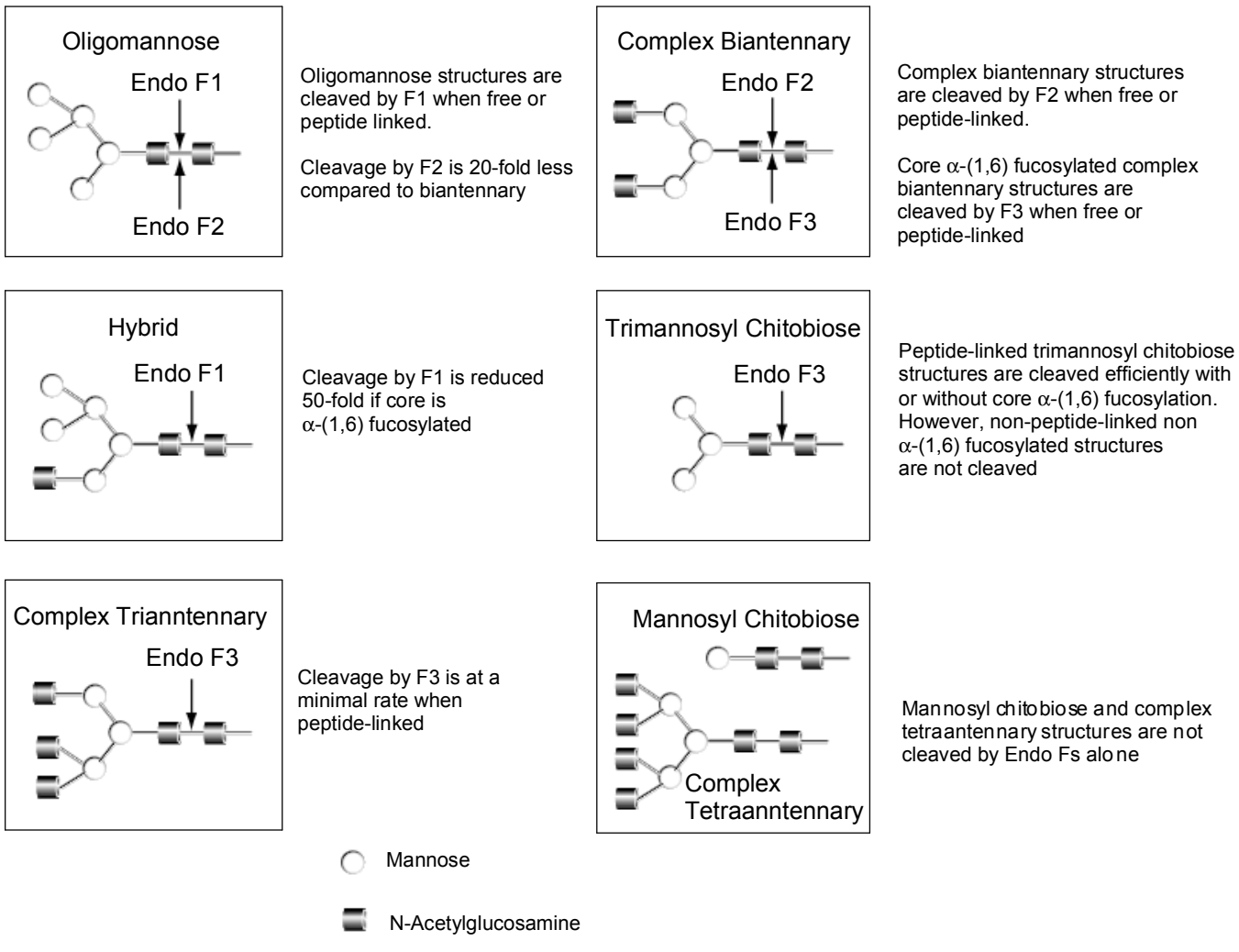


Figure 2.
Specificities of Endoglycosidases F1, F2, and F3 in conjunction with the EDEGLY Kit

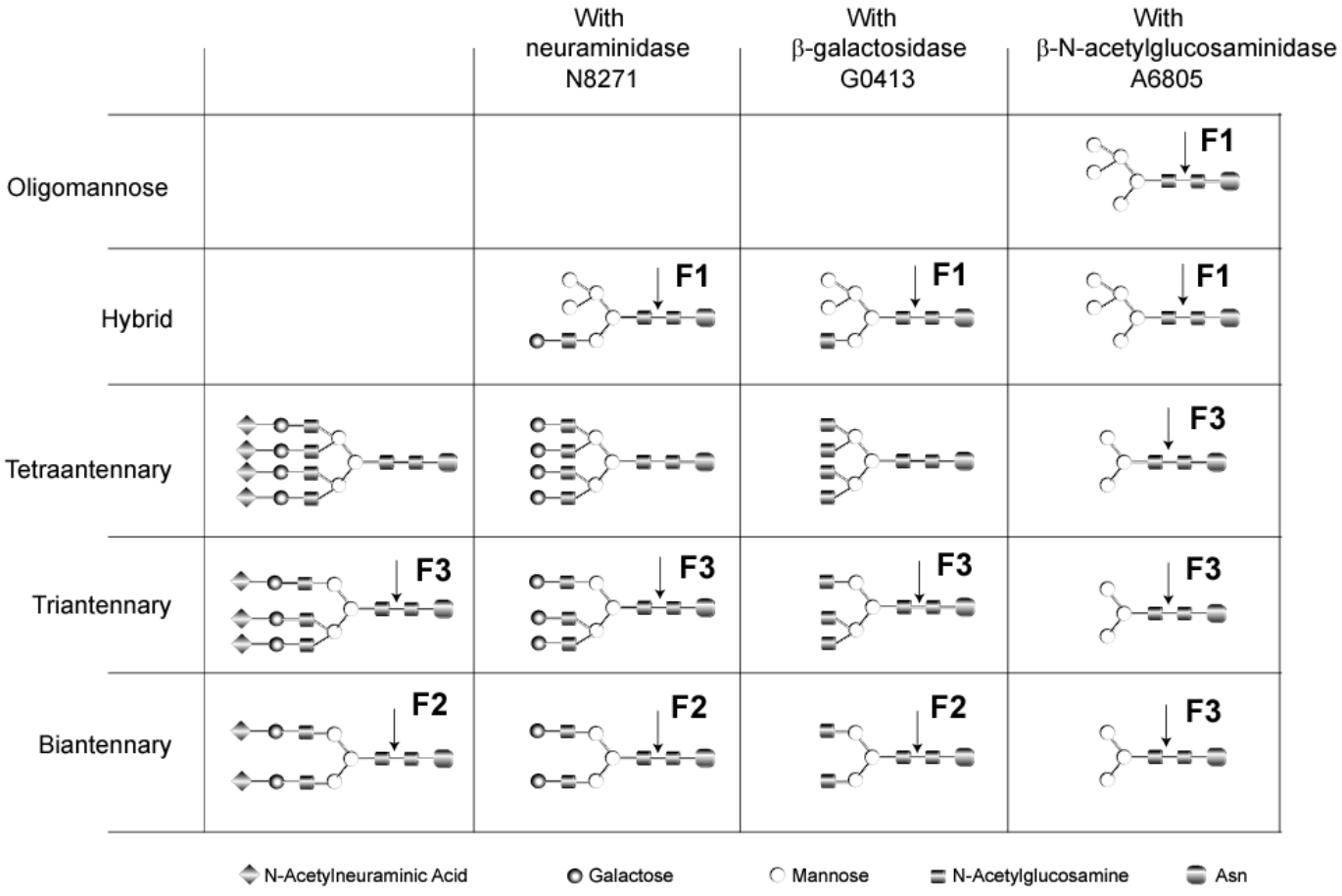


Figure 3.
Specificity of Engoglycosidase F1

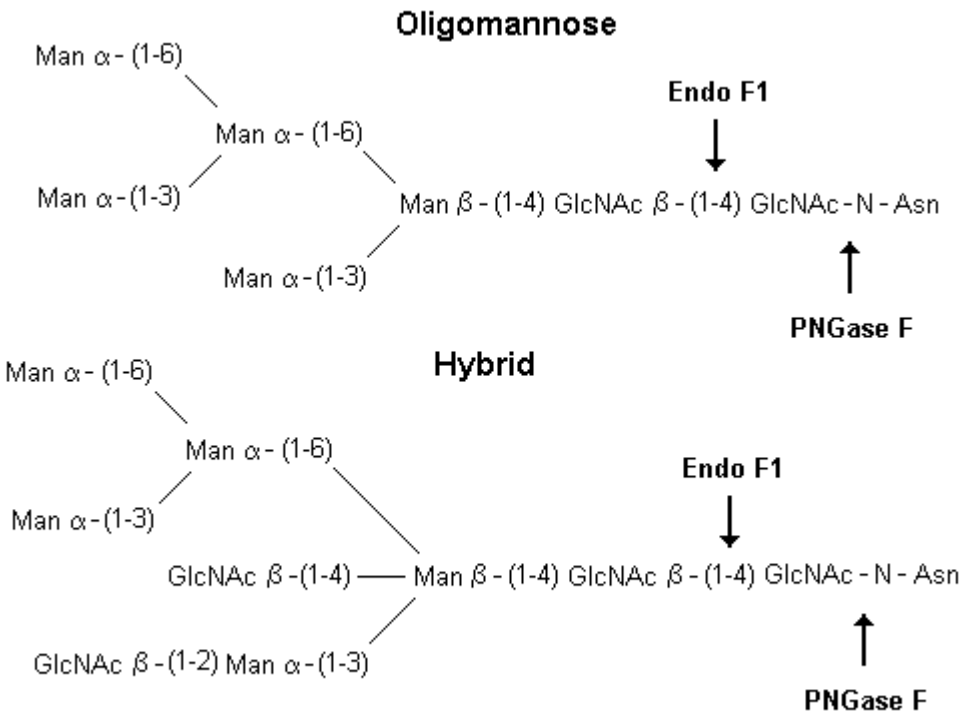
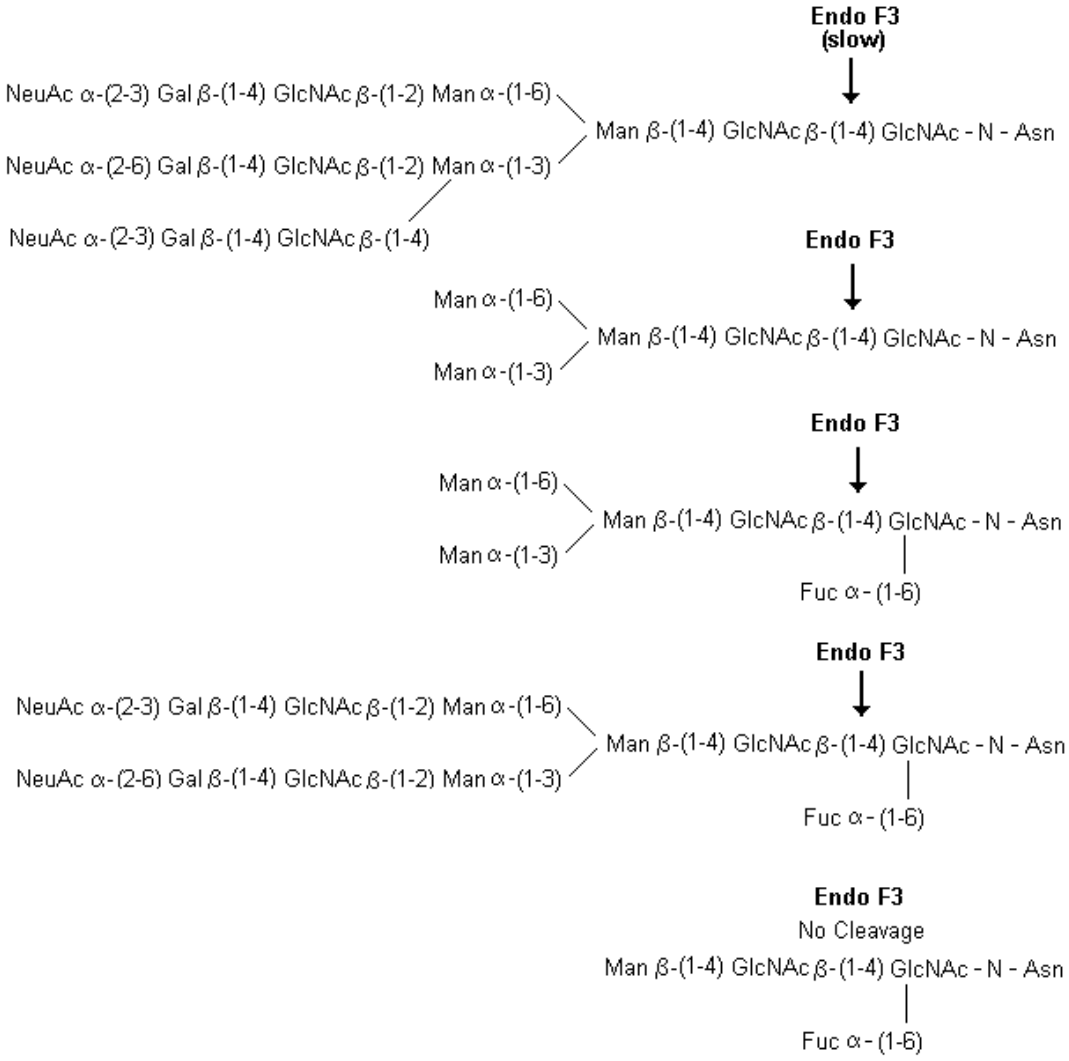


Figure 4.
Specificity of Endoglycosidase F3



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FF,GL,RBG,MAM 01/11-1