

Certificate of Analysis

Endo H:

Cat.# V4871

Part#	Component	Size
V487A	Endo H	10,000 units
V490A	10X Endo H Reaction Buffer	1ml
V492A	10X Denaturing Solution	1ml

Cat.# V4875

Part#	Component	Size
V487B	Endo H	50,000 units
V490A	10X Endo H Reaction Buffer	1ml
V492A	10X Denaturing Solution	1ml

Description: Endoglycosidase H (Endo H) is a recombinant glycosidase cloned from *Streptomyces plicatus* and overexpressed in *E. coli*. Endo H cleaves the chitobiose core of high-mannose oligosaccharides and a limited number of hybrid oligosaccharides from asparagine-linked glycoproteins. It does not cleave complex glycans. Enzymatic cleavage is between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, leaving one N-acetylglucosamine residue on the asparagine. This is in contrast to PNGase F, which cleaves all asparagine-linked oligosaccharides, with the exception of those that contain Fucose.

Molecular Weight: Endo H has a molecular weight of 29kDa.

Storage Conditions: Store at -30°C to -10°C.

Unit Definition: One unit is defined as the amount of enzyme required to remove >95% of the carbohydrate from 10µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10µl.

Physical Form: Endo H is supplied as a frozen liquid in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 5mM EDTA at a concentration of 500U/µl.

Reaction Buffer: 10X Endo H Reaction Buffer (Part# V490A) is composed of 0.5M sodium citrate (1X pH 5.5 at 25°C).

Denaturing Solution: 10X Denaturing Solution (Part# V492A) is composed of 5% SDS, 0.4M DTT.

Part# 9PIV487

Revised 3/13



Promega

Promega Corporation

2800 Woods Hollow Road		
Madison, WI 53711-5399		USA
Telephone	608-274-4330	
Toll Free	800-356-9526	
Fax	608-277-2516	
Internet	www.promega.com	

Quality Control Assay

This lot passes the following Quality Control specifications:

Purity: Purified to >95% as determined by SDS-PAGE analysis using Coomassie® blue detection.

Reference

1. Maley, F. *et al.* (1989) *Anal. Biochem.* **180**, 195-204.

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Signed by:

J. Stevens, Quality Assurance

1. SDS-PAGE Analysis of Protein Deglycosylation Using Endo H

1. Add 1–20µg of the target glycoprotein to an appropriate volume of water (or a compatible buffer at a low ionic strength) to a final volume of 9µl.
2. Add 1µl of 10X Denaturing Solution.
3. Heat the sample for 5 minutes at 95°C.
4. Allow the sample to cool to room temperature for 5 minutes.
5. Add 2µl of 10X Endo H Reaction Buffer and 1–5µl of Endo H (500–2500 units). Add enough water to increase the total volume of the reaction to 20µl. The reaction can be scaled up as needed.
6. Allow the reaction to proceed for 2–18 hours at 37°C.
7. Analyze the products using SDS-PAGE. Treated glycoproteins will appear to run at a lower molecular weight relative to untreated, control samples.

2. Mass Spectrometry Analysis of Protein Deglycosylation

This protocol avoids the use of detergents, which are incompatible with downstream MS analysis.

1. Add 1–20µg of the target glycoprotein to water, 25mM ammonium bicarbonate (pH 7.8) or ammonium acetate (pH 5.5) in a volume of 10µl.
2. Heat the sample for 5 minutes at 95°C.
3. Allow the sample to cool to room temperature for 5 minutes.
4. Add 2µl of 10X Endo H Reaction Buffer and 1–5µl of Endo H (500–2500 units) to the reaction. Add enough water to increase the total volume of the reaction to 20µl. The reaction can be scaled up as needed.
5. Allow the reaction to proceed for 2–18 hours at 37°C.
6. Samples are ready for MS analysis using either solution- or gel-based digestion protocols (1). To desalt the sample prior to mass spectrometric analysis, see the ZipTip® protocol given in the *Trypsin Gold, Mass Spectrometry Grade, Technical Bulletin #TB309*.

3. Related Products

Product	Size	Conc.	Cat. #
Asp-N, Sequencing Grade	2µg		V1621
Arg-C, Sequencing Grade	10µg		V1881
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endoproteinase Lys-C, Sequencing Grade	5µg		V1071
Fetuin	500µg	10mg/ml	V4961
Glu-C, Sequencing Grade	50µg (5 × 10µg)		V1651
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
PNGase F	500u	10u/µl	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5 × 1mg		V2072
Protein Deglycosylation Mix	20 reactions		V4931
rLys-C, Mass Spec Grade	15µg		V1671
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073

4. References

1. *Trypsin Gold, Mass Spectrometry Grade*, Technical Bulletin #TB309, Promega Corporation.