For life science research only.
Not for use in diagnostic procedures.

Endoglycosidase H

from *Streptomyces plicatus*, recombinant from *E. coli*, Endo-β-N-acetylglucosaminidase H

Version: 22

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Cat. No. 11 088 726 001	1 U 200 µl
Cat. No. 11 643 053 001	2.5 U 500 µl

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Endoglycosidase H	Solution in 50 mM sodium phosphate, 25 mM EDTA, 0.1% Micr-O-protect (w/v), pH 7.0.	11 088 726 001	1 vial, 200 µl
			11 643 053 001	1 vial, 500 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Endoglycosidase H	Store at +2 to +8°C. ⚠ ***Do not freeze; the enzyme will be inactivated.***

1.3. Additional Equipment and Reagent required

Incubation buffer

- 10 to 100 mM potassium buffer, pH 5 to 6, or
- 10 to 100 mM sodium acetate, pH 5 to 6, or
- 10 to 100 mM phosphate buffer, pH 5 to 6, or
- 10 to 100 mM sodium citrate buffer, pH 5 to 6
- 0.5 mM phenylmethylsulfonyl fluoride (PMSF*) or 0.5 M sodium rhodanide

For deglycosylation

- SDS*
- Triton X-100*
- Octylglucoside*

1.4. Application

Use Endoglycosidase H for the deglycosylation of glycoproteins.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Deglycosylation

The extent and rate of the deglycosylation of glycoproteins depend to a high degree on the nature of the glycoproteins. Therefore, no general instructions with regard to the incubation conditions can be given.

- The deglycosylation rate can be increased by denaturation of the glycoproteins, for example, by carboxymethylation, sulfitolysis, or by heating in the presence of sodium dodecyl sulfate* (SDS).
- In addition, this denaturation extends the hydrolysis also to such high mannose side chains, which are not hydrolyzed under native conditions.
- When using SDS, use a 1.2 fold excess of SDS in relation to the protein content of the incubation mixture.
- If the protein concentration is very low (<100 µg/ml), the SDS content should not exceed 0.02%, otherwise inactivation of the Endoglycosidase H may occur.
- The enzyme activity against glycoproteins containing inter- or intramolecular disulfide bridges is highly increased by addition of 0.1 M 2-mercaptoethanol.
- Detergents such as Triton X-100*, n-Octylglucoside*, or zwitterionic detergents show no influence on the enzyme activity.
- In general, 50 to 250 mU Endoglycosidase H should be sufficient to deglycosylate up to 1 mg high mannose glycoprotein/ml when incubated overnight.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Incubation buffer

10 to 100 mM potassium or sodium acetate, phosphate buffer, or sodium citrate buffer, pH 5 to 6.

⚠ ***When incubating under denaturating conditions overnight, add 0.5 mM phenylmethylsulfonyl fluoride (PMSF*) or 0.5 M sodium rhodanide in order to inhibit protease activity, for example, from the glycoprotein sample.***

2.2. Parameters

Contaminants

Absence of contaminants

α- and β-glucosidase, β-galactosidase, α-mannosidase, β-N-acetylhexosaminidase, and α-L-fucosidase:
After incubation of 500 mU Endoglycosidase H with the corresponding 10 mM 4-nitrophenyl glycosides for 17 hours at +37°C in 50 mM sodium acetate buffer, pH 5.5, in a final volume of 0.2 ml, no activities of the enzymes in question are found.

Proteases

After incubation of 500 mU Endoglycosidase H with 200 µg Universal Protease Substrate* (casein, resorufin-labeled) for 17 hours in 200 µl 50 mM Tris-HCl buffer, 5 mM calcium chloride, pH 7.8 at +37°C according to the method of Twinning, no protease activity is detectable.

EC-Number

EC 3.2.1.96

pH Optimum

The pH optimum of the enzyme is at pH 5 to 6.
Endoglycosidase H preferentially hydrolyzes N-glycans of the high mannose type.

Specific Activity

Approximately 40 U/mg enzyme protein.

Specificity

Active on N-linked oligosaccharides of glycopeptides/proteins. Cleaves only high mannose structures [n = 2, x and/or y = AcNeu-Gal-GlcAc].

Unit Definition

One unit is the enzyme activity which hydrolyzes 1 µmol dabsyl-Asn$(GlcNAc)_2(Man)_5$ or 0.2 µmol dansyl-Asn $(GlcNAc)_2(Man)_5$ within 1 minute at +37°C and pH 5.5.

3. Additional Information on this Product

3.1. Test Principle

Isolation and properties

Endoglycosidase H is isolated from a recombinant *E. coli* strain which carries the *Streptomyces plicatus* gene on a plasmid. Besides the Endoglycosidase H band, no other protein band is observed when analyzing 3 µg of enzyme by polyacrylamide gel electrophoresis.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Triton X-100	50 ml, 5 x 10 ml	11 332 481 001
n-Octylglucoside	10 g	10 634 425 001
Universal Protease Substrate	40 mg	11 734 334 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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