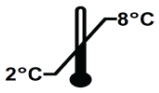
Leadgene® Endoglycosidase H (Endo H)

Removing glycans from glycosylated proteins using Endoglycosidase H to enhance the homogeneity of the target protein.

REF LGX-23001



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REF LGX-23001

※ **Please read and follow the instructions in this manual carefully before use.**

※ **For professional use only.**

1. Intended use

This product is for professional use only and is intended for use in the manufacturing process of biologics (including cell or gene therapy products) to enhance the yield and biological activity of the target protein/antibody.

2. Introduction and Principle

Endoglycosidase H (Endo H) is an enzyme with high specificity that functions to cleave mannose-containing oligosaccharide moieties from the peptide chain of glycoproteins, but it cannot cleave more complex oligosaccharides from glycoproteins. In eukaryotic cells, proteins undergo multiple glycosylation modifications during intracellular transport, which increases their diversity. However, this also complicates purification and subsequent applications. By utilizing Endoglycosidase H, oligosaccharide chains can be efficiently removed, leaving only a single N-acetylglucosamine sugar on the protein surface.

Using Leadgene® Endoglycosidase H helps improve the homogeneity of proteins expressed in eukaryotic cells, facilitates the purification process, and enhances the yield and stability of target proteins/antibodies.

3. Reagents provided

No. and items	Quantity/Spec.	Appearance	Status
LGX-23001 Leadgene® Endoglycosidase H	1 vial, 100 µL/vial, 500 U/µL	100 µL/vial in a yellow cap plastic transparent tube	Clarified liquid, ready-to-use
	1 vial, 1000 µL/vial, 500 U/µL	1000 µL/vial in a transparent cap plastic transparent tube	

4. Materials required but not provided

(1) LGX-23002 Leadgene® Endoglycosidase H (Endo H) Buffer Set: contains 10X glycoprotein denaturing buffer and 10X reaction buffer ***Recommended for use in combination with***

(2) High quality distilled water

(3) Adjustable micropipette

(4) Disposable microcentrifuge tubes

(5) Timer

(6) Vortex mixer

(7) Dry bath or heater, capable of heating up to 100°C

(8) Incubator capable of maintaining temperature at 37±1°C

(9) Vertical electrophoresis tank

(10) SDS-PAGE

(11) 5X SDS sample buffer

(12) SDS-PAGE running buffer

(13) SDS-PAGE staining buffer

(14) Disposable gloves

5. Reagent preparation

(1) All reagents should be freshly prepared, and any unused portion should be discarded at the end of the day.

(2) The specification of this product is 500 U/µL. Users should adjust the activity unit content as needed for the

reaction requirements before conducting the reaction test.

6. Storage and Shelf Life

(1) When unopened and stored at 2-8ºC, the reagent can be stored for at least 12 months. The expiration date is indicated on the outer packaging.

(2) Once opened, the product should be used within two weeks. Any remaining reagent should be refrigerated in 2-8℃ immediately after each use.

(3) The enzyme should be diluted and prepared on the day of use.

7. Precautions & warnings

(1) Used by professionally trained personnel only.

(2) Protective clothing and disposable gloves should be worn when using this product.

(3) Avoid direct contact with skin and eyes. In case of accidental contact, immediately rinse with plenty of clean water.

(4) For single use only.

(5) The instructions in the product manual must be followed when using this product.

(6) Do not use if expired or if there are any obvious abnormalities in appearance.

(7) Reagents from different batches/lots should not be mixed.

(8) All reagents should be thoroughly mixed before use.

(9) Use disposable pipette tips to avoid microbial contamination and cross-contamination between reagents.

(10) Experimental waste liquids should be disposed of in accordance with local regulations on medical biological safety waste management.

8. Procedures

※ This product is recommended to be used in combination with LGX-23002 Leadgene® Endoglycosidase H (Endo H) Buffer Set. Other suitable glycoprotein denaturation buffers or reaction buffers may also be selected based on actual needs.

(1) Pre-treatment of the target protein: Take 1-20 μg of the target protein, add 1 μL of GD23002 10x glycoprotein denaturing buffer from LGX-23002, and then add deionized water to bring the total reaction volume to 10 μL.

(2) Protein denaturation: Place the sample prepared in ***step (1)*** into a 100°C dry bath or heater and incubate for 10 minutes.

(3) Glycan removal reaction: Add 2 μL of RB23002 10x reaction buffer from LGX-23002 to the sample from ***step (2)***, then add the appropriate amount of LGX-23001 Leadgene® Endoglycosidase H as needed, and add deionized water to bring the total reaction volume to 20 μL. Incubate the reaction at 37°C for one hour in a constant temperature incubator.

9. Limitations

The bioactivity of this product may be influenced by the background buffer solution, pH, reducing agents, surfactants, and salt concentration of the target protein/antibody. It may also be affected by the quality of the glycoprotein denaturation buffer or reaction buffer used in combination with it.

10. Bibliographies/References

(1) Koide N, Muramatsu T. Endo-beta-N-acetylglucosaminidase acting on carbohydrate moieties of glycoproteins. Purification and properties of the enzyme from Diplococcus pneumoniae. J Biol Chem. 1974 Aug 10;249(15):4897-904.

(2) Pierce RJ, Spik G, Montreuil J. Cytosolic location of an endo-N-acetyl-beta-D-glucosaminidase activity in rat liver and kidney. Biochem J. 1979 Jun 15;180(3):673-76.

(3) Pierce RJ, Spik G, Montreuil J. Demonstration and cytosolic location of an endo-N-acetyl-beta-D-glucosaminidase activity towards an asialo-N-acetyl-lactosaminic-type substrate in rat liver. Biochem J. 1980 Jan 1;185(1):261-4.

(4) Tai T, Yamashita K, Ogata-Arakawa M, Koide N, Muramatsu T, Iwashita S, Inoue Y, Kobata A. Structural studies of two ovalbumin glycopeptides in relation to the endo-beta-N-acetylglucosaminidase specificity. J Biol Chem. 1975 Nov 10;250(21):8569-75. PMID: 389.

(5) Tarentino AL, Plummer TH Jr, Maley F. The release of intact oligosaccharides from specific glycoproteins by endo-beta-N-acetylglucosaminidase H. J Biol Chem. 1974 Feb 10;249(3):818-24. PMID: 4204553.

(6) Trimble RB, Maley F. Optimizing hydrolysis of N-linked high-mannose oligosaccharides by endo-beta-N-acetylglucosaminidase H. Anal Biochem. 1984 Sep;141(2):515-22. doi: 10.1016/0003-2697(84)90080-0. PMID: 6437277.

REF Catalogue number	Manufacturer
LOT Batch/Lot No.	+8 °C +2 Storage temperature
Consult instructions for use	Expiry date

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