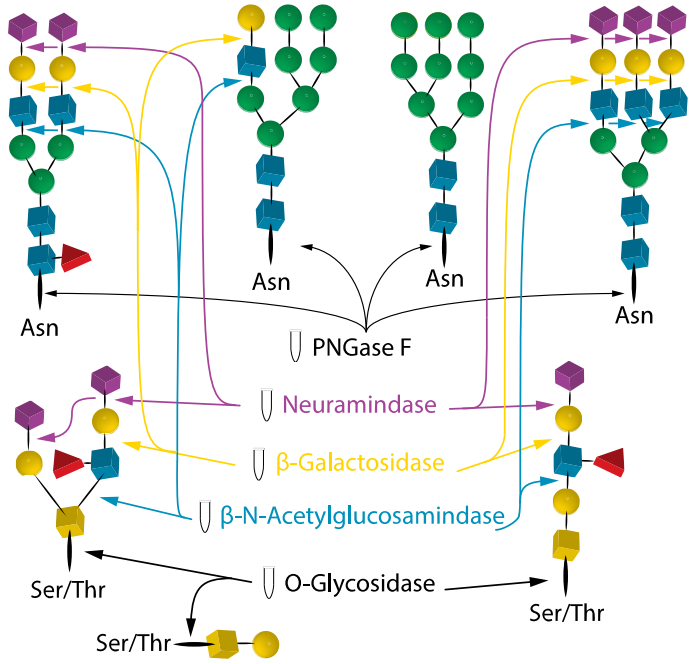

Enzymatic Deglycosylationm Kit

Part Number KE-DG01

Kit Storage Kits should be stored at 4°C.

Shipping This product should be shipped on frozen packs in an insulated container.

Kit Contents

Kit includes the enzymes, controls, and reagents required to remove all N-linked oligosaccharides and most O-linked sugars. Each kit will deglycosylate more than 2 mg of glycoprotein in 20 reactions.

Enzymes

20 µLs of each of the following enzymes in separate vials:

- PNGase F (*E. meningosepticum*)
- O-Glycosidase (*S. pneumoniae*)
- Neuraminidase (*A. ureafaciens*)
- ß-Galactosidase (*S. pneumoniae*)
- ß-N-Acetylglucosaminidase (*S. pneumonia*)

refer to enzyme specifications for further details

Other Supplied Reagents

- 5x Reaction buffer - 200 µL
  - 250 mM sodium phosphate, pH 7
- Denaturation Solution - 100 µL
- Triton X - 100 µL
- Bovine Fetuin (control) - 10 mg/ml

Control

Fetuin is include in this kit as a positive control of the deglycosylation reaction. The concentration of the fetuin is 10 mg/ml .The molecular weight is approximately 48,000 daltons.

Specificity

The Enzymatic Deglycosylation Kit will remove all N-linked oligosaccharides and many O-linked oligosaccharides from glycoproteins. N-links (Asn-linked) are removed using the enzyme PNGase F. In addition, all Ser/Thr-linked (O-linked) Gal-(β1-3)-GalNAc-(α1) and all sialic acid substituted Gal-(β1-3)-GalNAc-(α1) will be removed using the combination of Neuraminidase and O-Glycosidase. The addition of ß-Galactosidase and ß-N-acetylglucosaminidase will assist in the deglycosylation of larger O-link structures.

KE-DG01 Enzymatic Deglycosylation Kit

Specifications - Protocol

QA-Bio
www.QA-Bio.com
info@QA-Bio.com

phone (Asia) +65 3158 9855
phone (Europe) +48 22 104 0892
phone (USA) +1 760 249 2664

Denaturing Protocol

1. Dissolve 100 µg or less of a glycoprotein in 30 µL deionized water in an Eppendorf tube.
2. Add 10 µL 5X Reaction Buffer 7 and 2.5 µL Denaturation Solution. Mix gently.
3. Heat at 100°C for 5 minutes.
   NOTE: Some proteins may precipitate when heated with SDS. In this event, omit the heat treatment and increase the incubation time to 24 hours after adding enzymes.
4. Cool to room temperature. Add 2.5 µl Triton X-100 solution. Mix gently.
   NOTE: Failure to add Triton X-100 may result in the reduction of activity of some enzymes.
5. Add 1 µL each of each enzyme.
6. Incubate for 3 hours at 37°C.
7. Analyze by method of choice.

Alternatively, the enzymes may be added individually or sequentially in order to determine what types of oligosaccharides are present on the glycoprotein.

Non-denaturing Protocol

1. Dissolve 100 µg or less of a glycoprotein in 35 µL deionized water in an Eppendorf tube.
2. Add 10 µL 5X Reaction Buffer 7.
3. Add 1 µL of each enzyme.
4. Incubate for 1-5 days at 37°C.

An aliquot should be deglycosylated using the denaturing protocol to provide a gel standard for the fully deglycosylated protein. The position of the native protein can then be compared with this standard to judge the extent of deglycosylation.

Warranties and liabilities

QA-Bio warrants that the above product conforms to the attached analytical documents. Should the product fail for reasons other than through misuse QA-Bio will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose. QA-Bio shall not be liable for any incidental, consequential or contingent damages. This product is intended for *in vitro* research only.

updated January 29, 2025

QA-Bio
www.QA-Bio.com
info@QA-Bio.com

phone (Asia) +65 3158 9855
phone (Europe) +48 22 104 0892
phone (USA) +1 760 249 2664