Enzymatic CarboRelease™ Kit

Part Number KE-DG01

Certification of Analysis Lot Number 505.1C
Kit Storage Kits should be stored at 4°C.

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
PNGase F (*C. meningosepticum*)
20 µL - 100 mU
O-Glycosidase (*S. pneumoniae*)
20 µL - 25 mU
Sialidase (*A. ureafaciens*)
20 µL - 100mU
ß-Galactosidase (*S. pneumoniae*)
20 µL - 1 U
Glucosaminidase (*S. pneumonia*)
20 µL - 20mU
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 CarboRelease 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 Sialidase and O-Glycosidase. The addition of ß-Galactosidase and Hexosaminidase will assist in the deglycosylation of larger O-link structures.

Directions for Use
1. Mix 10 µl of 5x reaction buffer with up to 100 µg of glycoprotein in 35 µl distilled water in a 1.5 ml tube.

2. Add 2.5 µl denaturation solution. Mix gently and place in boiling water bath for 5 minutes. Chill on ice.

3. Add 2.5 µl of Triton-X.

4. Add 1 µl each of PNGase F, Sialidase, ß-Galactosidase, Glucosaminidase, and O-Glycosidase. Incubate for 3 hours at 37°C.

Note: Denaturation increases the rate of enzyme digestion up to 10 fold. If denaturation is not desired omit step 2-3, add with 5 µl of distilled water and increase incubation time to 24 hours.

The efficiency of deglycosylation can be tested by running a sample on a SDS-PAGE gel.

 updated 6/30/05

KE-DG01 CarboRelease Kit

Specifications - Protocol

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