QA-Bio CarboSeq™ N Kit

N-linked Oligosaccharide Sequencing Kit

Catalog Number
KE-SQ01

Certification of Analysis Lot Number:
401.1A

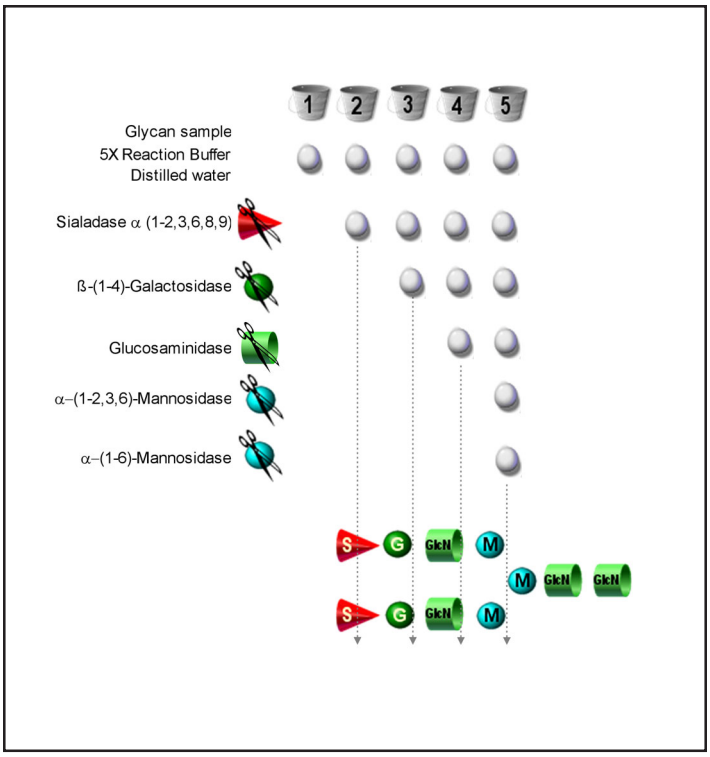
Application
Kit includes the enzymes and buffers required to sequence ten isolated, N-linked oligosaccharides. Most analysis techniques require labeling of the glycans prior to enzymatic digestions with a flourescent molecule such as 2-AB or ANTS.

Storage
Store kit at 4°C. Do not freeze.

Stability
Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Components
- Sialadase Alpha (1-2,3,6,8,9) (*A. ureafaciens*) - 80 µl
- ß-(1-4)-Galactosidase (*S. pneumoniae*) - 60 µl
- ß-Glucosaminidase (*S. pneumoniae*) - 40 µl
- α-(1-2,3,6)-Mannosidase (Jack Bean) - 20 µl
- α-(1-6)-Mannosidase (*X. manihotis*) - 10 µl
- 5X Reaction Buffer - 400 µl pH 5

Sample Preparation
Typically, glycan samples are isolated from bands excised from a profiling gel or fractions collected from a HPLC profile.



Purity
All QA-Bio Enzymes are tested for contaminating protease by incubating 10 µg of denatured BSA with 2 µl of enzyme at 37°C for 24 hours. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strains for our recombinant enzymes have been extensively tested and do not produce any detectable glycosidases. Enzymes purified from native sources are tested for contaminating exoglycosidases The absence of exoglycosidase contaminants is confirmed by extended incubations with the corresponding pNP-glycosides.

Protocol

KE-SQ01 CarboSeq N
Specifications - Protocol

QA-Bio
240 Eaton Road
San Mateo, CA 94402

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

866-384-2272
650-347-9700
fax 650-649-2394

Add components of the enzyme reaction to a series of six tubes as indicated in the following table.

Note: Prior to addition of α-(1-6)-Mannosidase to tube 5, incubate all tubes overnight at 37°C. Following overnight incubation, add 2 µl of α-(1-6)-Mannosidase to tube 5 and incubate 30 minutes at 37°C. If included in the initial incubation, α-(1-6)-Mannosidase will inhibit α-(1,2,3,6)-Mannosidase .

	Reaction Tube				
	1	2	3	4	5
Glycan Sample	2	2	2	2	2µl
5x Reaction Buffer	4	4	4	4	4µl
Distilled water	14	12	10	8	6µl
Sialadase		2	2	2	2µl
β-Galactosidase			2	2	2µl
β-Glucosaminidase				2	2µl
α-(1,2,3,6)-Mannosidase					2µl
	Incubate overnight at 37°C				
α-(1-6)-Mannosidase					2µl
	Incubate 30 minutes at 37°C				

If core fucosylation is suspected, an additional overnight incubation with α-(1-6)-Fucosidase (not included in kit - part number E-F006) is required.

Another common modification of human N-linked oligosaccharides is an α-(1-3,4) linked fucose attached to the N-acetylglucosamine on one or more antennae(Lewis X structure). The fucose will block cleavage by ß-galactosidase and ß-glucosaminidase, observed as an incomplete digestion by these enzymes. Inclusion of α-(1,3) fucosidase (E-F002 -not included) with the ß-galactosidase will permit complete digestion of the oligosaccharide and provide evidence for this important modification.

Analysis

Many methods of analysis are available, including HPLC, gel electrophoresis, HPAEC, capillary electrophoresis, and mass spectrometry. For more information on these methods, please contact us.

References

Dwek, R. A., C. J. Edge, D. J. Harvey, M. R. Wormald and R. B. Parekh. Analysis of glycoprotein-associated oligosaccharides. Ann Rev Biochem 62: 65-100 (1993).

Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates . Anal Biochem 100: 1-14 (1979).

O'Neil Enzymatic Release of Oligosaccharides from Glycoproteins for Chromatographic and Electrophoretic Analysis. J Chromatogr A . . . 720:1-2, 201-15(1996).

Rudd et al. Oligosaccharide Sequencing Technology. Nature. 388: 6638, 205-7(1997) .

Schaumann et al. Analytical Technique for Studying the Structure Of Glycoprotein N-Glycans. J. of Chromatography. 646: 227-234(1993) .

Jackson. The Analysis of Fluorophore-Labeled Carbohydrates by Polyacrylamide Gel Electrophoresis. Mol. Biotechnol. 5:2 101-23(1996) .

Starr et al. Fluorophore-Assisted-Carbohydrate- Electrophoresis, FACE, In The Separation, Analysis, and Sequencing of Carbohydrates. J Chromatogr A. 720:295-321 (1996) .

Prime, S., et al. Oligosaccharide sequencing based on exo- and endoglycosidase digestion and liquid chromatographic analysis of the products. J Chromatogr A 720: 263-274 (1996).

Anumula, K.R. & Du, P. Characterization of Carbohydrates using Highly Fluorescent 2- Aminobenzoic acid Tag Following Gel Electrophoresis of Glycoproteins. Anal. Biochem. 15:236-242(1999).

Harvey DJ. Matrix-assisted laser desorption/ionisation mass spectrometry. J Chromatogr A. 720(1-2):429-46(1996).

Geyer Het al. Structural analysis of glycoconjugates by on-target enzymatic digestion and MALDI-TOF-MS.
Anal Chem. 71(2):476-82(1999).

Warranties and liabilities

QA-Bio warrants that the above product conforms to the specifications herein. 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 August 2, 2004

QA-Bio
240 Eaton Road
San Mateo, CA 94402

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

866-384-2272
650-347-9700
fax 650-649-2394