



QA-Bio CarboSeq™ N Kit

N-linked Oligosaccharide Sequencing Kit

Catalog Number

KE-SQ01

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 fluorescent molecule such as 2-AB or ANTS.

Storage

Store kit at 4°C. Do not freeze.

Stability

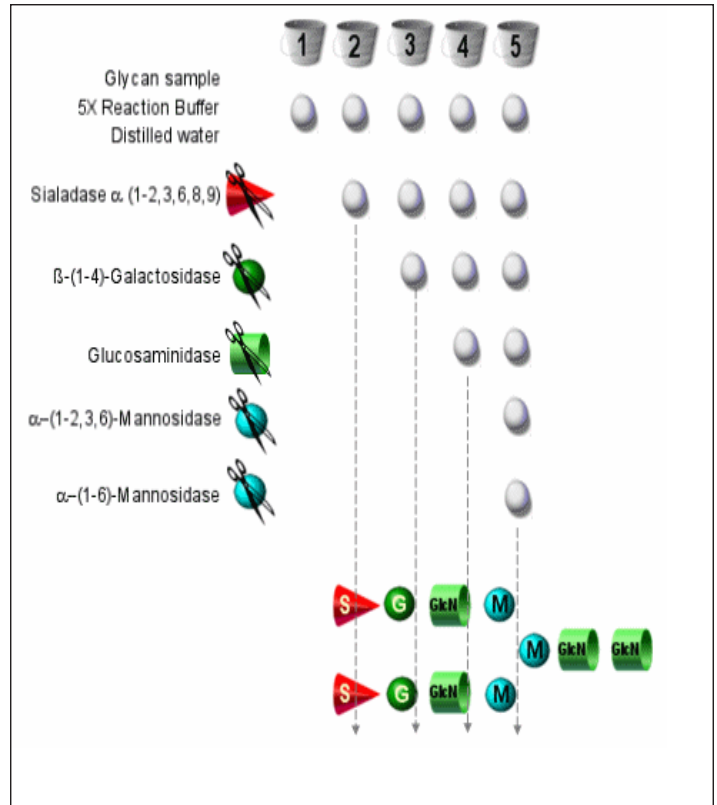
Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Components

- Sialidase Alpha (1-2,3,6,8,9) (*A. ureafaciens*) - 80 µl
- β-(1-4)-Galactosidase (*S. pneumoniae*) - 60 µl
- β-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.

KE-SQ01 CarboSeq N
Specifications - Protocol

Protocol

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 μ l 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	2 μ l
5x Reaction Buffer	4	4	4	4	4 μ l
Distilled water	14	12	10	8	6 μ l
Sialidase		2	2	2	2 μ l
β -Galactosidase			2	2	2 μ l
β -Glucosaminidase				2	2 μ l
α -(1,2,3,6)-Mannosidase					2 μ l
α -(1-6)-Mannosidase					2 μ l
	Incubate overnight at 37°C				
	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 β -galactosidase and β -glucosaminidase, observed as an incomplete digestion by these enzymes. Inclusion of α -(1,3) fucosidase (E-F002 -not included) with the β -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

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Warranties and liabilities

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