Carbohydrate Analysis/Detection

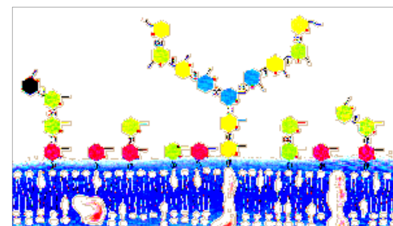
Product Description

Name : **Carbohydrate Analysis/Detection Kit**

EDANS based

Catalog Number : FP-CG4891, 1 kit (V)

Contains:
CG489a: fluorescent reagent (1,5-EDANS), 2.0 mL, 20 mM solution (M)
CG489b: reduction reagent (Sodium cyanoborohydride), 2ml, 1.0M solution in DMSO (K)



Storage: Fluorescent reagents and fluorescently labeled oligosaccharides should be handled with care, kept cold (ice bath) when not in use, and stored at 4°C. In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition.

Introduction & Overview

Numerous native proteins contain post-transcriptional glycosidic elaboration (sugars) whose structures are dependent both on species and cell type [1]. The characterization of the complex oligosaccharides obtained from these glycoproteins has proven a difficult and time consuming endeavor [2,6].

The Carbohydrate Analysis/Detection Kit is capable of quickly estimating and/or comparing the composition of the carbohydrates in such samples. The Kit provides reagents and protocols for analyzing these carbohydrates through covalent labeling with a fluorescent reagent (1,5-EDANS), and analysis .

The principle involves **enzymatic removal** of the oligosaccharides from a native protein (or mixture of reducing sugars), reductive amination of the reducing sugars and analysis of the resultant glycamines using silica-gel two dimensional thin layer chromatography (**2D-TLC**) or by other well established techniques [2,6].
For more sensitive analysis and comparison of oligosaccharides, polyacrylamide gel electrophoresis (**PAGE**) of labeled oligosaccharides can be used.

This technique, used in combination with various methods of enzymatic release and degradation of N- and O-linked oligosaccharides, can be used for a variety of analytical processes, described briefly below. The monosaccharide composition of a sample can be determined by release of oligosaccharide from the glycoprotein, followed by complete hydrolosysis to produce monosaccharides which are subsequently fluorescently labeled and analyzed by PAGE. Oligosaccharide profiles of glycoproteins can be determined by enzymatic release of N- or O-linked oligosaccharides, followed by labeling and electrophoresis of the intact oligosaccharide against known standards. **Sequencing** of N-linked oligosaccharides can be accomplished by isolation of oligosaccharide from a profiling gel, then subjecting the oligosaccharide to systematic digestion by exoglycosidases with known, highly specific substrate specificity. The resulting digestion products are analyzed by PAGE to determine the sequence of the oligosaccharides.

The **advantages of using the 1,5 EDANS** fluorophore include its low detection limit, water solubility, pH fluorescence invariance, stability, distinctive fluorescence from protein chromophores, and ability to be detected using normal phase chromatography techniques. The 1,5-EDANS labeling reagent also has advantages over the commonly used ANTS reagent: The more nucleophilic primary amine of 1,5-EDANS makes it more reactive in the labeling reaction than the aromatic amine of ANTS. In addition, 1,5-EDANS is less polar than ANTS, having only one charged sulfate group instead of three, allowing wider potential application for a variety of carbohydrate sizes.

We anticipate the use of this new kit to also aid other carbohydrate analyses including HPLC detection and purification, carbohydrate receptor analyses, enzyme inhibition studies, and the like. Additional information on these techniques is available. Custom couplings and determinations can also be arranged if desired. Please contact our technical services department for further information.

Directions for use

Materials

A/ Fluorescent Reagent. The 1,5-EDANS fluorescent reagent #CG489a (2.0 mL) is a 20 mM solution in .1N NaHCO3, pH 10 with NaOH.
B/ Reduction Reagent. The Sodium cyanoborohydride reduction reagent #CG489b (2.0 mL) is prepared with DMSO #CG489c to give a 1.0 M solution.

Handling and Storage

–20°C for component #CG489a (M) – Room temperature for others (Z)
Fluorescent reagents and fluorescently labeled oligosaccharides should be handled with care, kept cold (ice-bath) when not in use, and stored at 4°C. In case of contact with skin or eyes, wash thoroughly with soap and cold-water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition.

Protocol -A- ENZYMATIC RELEASE OF OLIGOSACCHARIDES FROM GLYCOCONJUGATES

Hydrazinolysis has been the most commonly used method to release N-linked oligosaccharides from glycoproteins. However the hydrazinolysis reaction can cause concurrent N-acyl removal from N-acetylglucosamine residues, and other unexpected side reactions [3]. We therefore suggest **enzymatic removal** of the carbohydrate side chains from appropriately purified proteins, typically using N-oligosaccharide glycopeptidase (EC 3.5.1.52)(Seikagaku Kagyo, Tokyo) [3] or N-glycanase (peptide N-(N-acetyl-ß-glucosaminyl)asparagine amidase) (Genzyme Corp., Boston, USA) for Asn-linked (N-linked) carbohydrates, or O-Glycanase (endo-b-Nacetylgalactosaminidase (EC 3.2.1.97) (Genzyme Corp., Boston 1-800-332-1042) for Ser/Thr O-linked carbohydrates [4]. In addition, several other enzymes are also available for release of oligosaccharides from glycolipids (Endoglycoceramidase) or for the release of the highly charged sialic acid (neuraminic acid) terminal sugars from the oligosaccharides prior to analysis (neuraminidase, EC 3.2.1.18).

Typical reaction conditions are as follows:

A.) N-Linked Oligosaccharide Hydrolysis.

Incubate 20 µg glycoprotein (denatured with SDS) with 0.03 - 0.3 units N-Glycanase enzyme in a total reaction volume of 30 µL overnight at 37°C, pH 7.0 - 8.6. Approximately 1.8 units of enzyme are needed for 20 µg native glycoprotein.

B.) O-Linked Oligosaccharide Hydrolysis.

Prior removal of terminal sialic acid residues (neuraminidase) is suggested. Generally 20 µg or native glycoprotein (or 100 µg of glycopeptide) are treated with 4 milliunits of enzyme in 0.25 M sodium phosphate buffer (pH 8.4) (100 µL) for 6 hours at 37°C. Purification of the resultant oligosaccharides is performed by gel-filtration chromatography (Bio-Gel P-4, 200-400 mesh, elution with distilled water), or as described in section VIII below. Identification of the carbohydrate containing fractions is carried out using orcinol-sulfuric acid analysis.

Protocol -B- FLUORESCENT LABELING

The standard method of reacting released oligosaccharides (or standards) with the fluorescent reagent 1,5 - EDANS involves placing suitable volumes (typically 50- 100 µL) of approximately 1.0 mM saccharide solutions in water in microcentrifuge tubes and freeze-drying in a centrifugal vacuum evaporator.

To each lyophilized sample is added 5 µL of 20 mM 1,5-EDANS solution in 0.1 N NaHCO3, pH 10 (Solution A) and 5 µL 1.0 M Sodium cyanoborohydride solution in DMSO (solution B).
NOTE: Prepare solution B by mixing the sodium cyanoborohydride reagent (CG489b) with the Dimethylsulfoxide reagent (CG489c) thoroughly just prior to use, in the vial provided, to give a 1.0 M sodium cyanoborohydride solution.

Vortex mix reactions and centrifuge briefly to ensure all reactants are in the tips of the tubes. Incubate reaction at 37°C overnight (15 hr).

Dry the reactions in a centrifugal vacuum evaporator and redissolve at a suitable concentration in the appropriate TLC solvent or electrophoresis sample buffer [5]. For TLC analysis, a final concentration of 1.0 nmol/µl works well. For electrophoresis, a final concentration of 100pmol/µl is recommended.

Protocol -C- THIN LAYER CHROMATOGRAPHIC ANALYSIS

Silica gel 60 plates (5.0 x 5.0 cm; 0.2 mm thickness.) were spotted with 5 µL of the fluorescently labeled saccharide at a position of approximately 5 mm above one edge of the plate. Elutions of the plate (in two-dimensions) is carried out in an appropriate TLC chamber, preequilibrated with solvent, using one of the following irrigants: n-Butanol:Formic Acid:Water (4:6:1); Ethyl Acetate:Methanol:Acetic Acid (8:2:0.2); 2-Propanol:Water (8:2); 95% Ethanol; Ethyl Acetate: Methanol: Water: Acetic Acid (7:1:1:1); or other appropriate combinations of these. In addition, other irrigant systems may be developed by the user.

Detection of the resulting bands can be made using an ultraviolet light source (hand-held UV light source, Mineralight, UVP, Inc.), with excitation at 365 nm (long-wavelength). Comparison of the resultant bands with those obtained from standards, produced under the same conditions and developed on the same plate will enable identification of the unknown carbohydrates.

Protocol -D- PAGE ANALYSIS

The labeled saccharides may be subjected to PAGE using a type SE600 electrophoresis apparatus from Hoefer Scientific Instruments, or equivalent. The electrophoretic buffer is based on the Tris/HCl glycine discontinuous system Laemmli [5], but with SDS omitted throughout.
The polyacrylamide gel consists of a linear gradient from 20 to 40% as described by Jackson [2]. 2 µL of the labeled saccharide solution in buffer (100pm/µl) is electrophoresed at 100V for 30 min, 500V for 30 min, and finally at 1000V for 120 minutes, or until the buffer front reached 5-10 mM from the gel base. Gels may be viewed or photographed using a U.V. light box with a maximum emission wavelength of 302 nm.

Protocol -E- HPLC ANALYSIS

HPLC systems using either Spherogel TSK DEAE-2SW analytical ion-exchange (4.6 x 250mm; Beckman Instruments Inc., San Ramon, CA), Supelcosil LC-Si, 5um (4.6 x 25 cm; Supelco, Inc., Bellefonte, PA); LiChrosorb Si60, 10um (E. Merck and Co., Darmstadt, Germany) or Spheracil C-18 SC2.1/10 (Pharmacia Biotech, Milwaukee, WI) can be used to analyze the EDANS derivatized glycamines [7-9]. Samples (5-10uL) are injected onto the column. Flow rates are 0.5 - 1.0 mL/min. Solvent systems consist of gradient acetonitrile:water (90-50%). Octadecylamine can be added as an in-situ ion-exchange reagent for silica-based columns. Samples are detected at 254, 280 or 336 (lambda max) nm (UV-detection) or at 495 nm (fluorescence detection).

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Protocol -F- GEL FILTRATION CHROMATOGRAPHIC ANALYSIS

Gel filtration analysis of the derivatized carbohydrates is carried out using Bio-Gel P-2, or P-4 columns [10-11] using water or 10%ethanol/water as eluent. Detection of the resulting separated bands can be accomplished on the column using a UV-light source (long wavelength, 360 nm excitation) and by analysis of the eluted fractions as described above.

REFERENCES

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5.) Laemmli, U. K., Nature (London) 227 (1970) 680-685.
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Disclaimer

The above information is given as a viable methodology for use of the Carbohydrate Analysis/Detection Kit.
The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.
These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited.
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