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ProductInformation

GlycoProfile® 2-AA Labeling Kit

Product Code **PP0530**
Store at Room Temperature

TECHNICAL BULLETIN

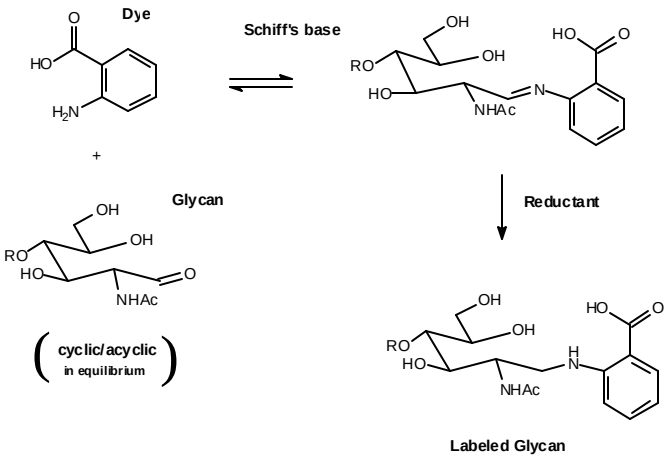
Product Description

Glycan analysis has become an increasingly critical aspect of glycomics and proteomics, as the role of glycoproteins in cell signaling, cell adhesion, immune response, and disease states is made clear through ongoing research. Because glycans tend to have low spectral activity in both UV and visible light, it is often necessary to label them in order to enhance detection.

The fluorophores 2-AB (2-aminobenzamide) and 2-AA (anthranilic acid or 2-aminobenzoic acid) provide valuable tools for glycan analysis, due to their sensitivity and stability when bound to glycans.[1] Samples containing mixed pools of glycans can often be detected at picomolar concentrations. While 2-AB is somewhat less sensitive than 2-AA, it is suitable for downstream glycan analysis by HPAE (high-performance anion-exchange), HPLC, and ESI-MS (electrospray mass spectroscopy) methods.[2-8] Although suitable for the same applications, 2-AA is better suited for SDS-PAGE.

Glycan binding to both dyes is very robust with no degradation during post-cleanup analysis. Labeling can be performed on either purified or pooled samples, including a variety of sources, such as N-linked, O-linked, and GPI anchored glycans. For samples containing sialated oligosaccharides, sialic acid loss is negligible. Other commonly used methods such as radiolabels, antibody labels, and various probes do not display the stability, flexibility, and ease of use observed with 2-AA and 2-AB.

Glycans with a free reducing sugar exist in equilibrium between the cyclic (closed ring) and acyclic (open ring) structures. A stable Schiff's base is formed when the carbonyl carbon of an acyclic reducing sugar is linked to the amine moiety of the dye in a nucleophilic manner. Following formation of the Schiff's base, the resulting imine group is reduced using sodium cyanoboro-hydride, resulting in a stable labeled glycan.



Components

The kit contains sufficient reagents for labeling up to 36 samples. Two sets of components have been provided. Each set is sufficient for labeling up to 18 samples based on a 5 µl reaction volume (to accommodate use with the GlycoProfile Glycan Clean-up Cartridges, Product Code G 8169). Prior to the clean up step, larger samples can be split accordingly.

Mixed glycan samples should contain between 100 picomoles to 50 nanomoles of purified glycans. With a single pure glycan, as little as 5 picomoles may be labeled and detected in subsequent HPLC analysis.

2-AA (Anthranilic Acid) (Product Code A 6729)	2 X 6 mg
DMSO (Dimethyl sulfoxide) (Product Code D 4942) 350 µl per vial	2 X 1 vial
Acetic Acid, Glacial (Product Code A 9353)	2 X 200 µl
Reductant (Sodium Cyanoborohydride) (Product Code R 5153)	2 X 6 mg

Reagents Recommended but Not Provided
GlycoProfile Glycan Clean-Up Cartridges
(Product Code G 8169)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended that the entire Technical Bulletin be read prior to starting the procedure.

Storage/Stability
The kit, as supplied, is stable for at least one year when stored unopened at room temperature. The 2-AA reagent (anthranilic acid, Product Code A 6729) is light sensitive and must be stored in the dark. Labeled glycans should be stored at –20 °C in the dark. For optimal results, the labeling reagent should be used within one hour of component reconstitution and preparation.

Procedure
Glycan samples may be prepared by enzymatic deglycosylation or chemical deglycosylation via hydrazinolysis. For mixed pools of glycans, the optimal sample size is 100 pmole to 50 nmole. For purified samples of a single glycan, much lower amounts may be labeled. As little as 5 pmoles of a single purified glycan be detected in post-labeling analysis by HPLC.

1. Prior to labeling, glycan samples should be purified to remove protein, peptides, salts, detergents, and any additional contaminating substances.
2. Transfer the glycan sample solutions into the reaction vials and dry completely. Excess moisture will have negative effects on labeling and stability.
   a. Optimal results are obtained when samples are dried by centrifugal evaporation. Use caution with lyophilization. Ensure that each sample dries as a concentrated mass at the bottom of the vials.
   b. Do not expose samples to extreme pH or high temperatures.
      i. Temperatures greater than 28 °C and/or low pH will result in desialylation.
      ii. Exposure to high pH may result in epimerization of reducing sugars.
3. Prepare the Labeling Solution immediately prior to labeling.
   a. Tap or briefly spin down component vials to avoid loss of reagents in the cap or on the walls of ampules. To open ampules, hold both the body and the top of the ampule, then gently, but firmly, snap open at the colored break-ring, directing the break away from your body.
   b. Add 150 µl of Acetic Acid, Glacial (Product Code A 9353) to the entire ampule of DMSO (Product Code D 4942). Note that DMSO forms a semi-solid at cooler temperatures, hence warming may be required.
   c. Add 100 µl of the DMSO/Acetic Acid mixture (step 3b) to the entire vial of 2-AA (Product Code A 6729). Mix until dissolved; vortexing may be required.
   c. Add the entire volume from step 3c to the vial of Reductant (Product Code R 5153). Mix until completely dissolved. If insoluble particulates remain, the solution may be heated to 65 °C for up to 3 minutes. Any remaining particulates can be dissolved by adding 10 µl of water, (HPLC grade or better). This is the Labeling Solution and should be protected from light. It is stable for 1 hour.
4. Add 5 µl of the Labeling Solution (step 3d) to each dried glycan sample. Cap and mix thoroughly. Tap or spin down to collect the dissolved sample in the bottom of each vial.
5. Incubate glycan samples for 3 hours at 65 °C in a heating block, dry oven, or sand tray. Avoid moist heat if possible. If a water bath or moist heat is used, the vials must be tightly sealed.
   Note: Incubation periods of between 2 and 4 hours will not significantly affect the final results.
6. If insoluble particulates are present, vortex the pre-heated samples from step 5 for 30 minutes.
7. Following incubation, briefly spin the vials to re-collect each sample at the bottom of the reaction vial. Allow the samples to cool to ambient temperature prior to proceeding with post-labeling analysis.
   a. Extended periods of time between incubation and analysis may result in desialylation of labeled glycans and consequently should be avoided.
   b. Sample clean up using GlycoProfile Glycan Clean-Up Cartridges (Product Code G 8169) is recommended to remove excess dye and labeling reagents. A purification protocol is included with the cartridges.

Analysis of 2-AA labeled glycans

The 2-AA reagent has an excitation range of 250-375 nm, with a peak excitation at 315 nm. The emission range for 2-AA is 275-800 nm, with a peak emission at 400 nm.

Once the glycans have been labeled a variety of methods exist to analyze them. The most common techniques employ fluorescent detection after separation by HPLC or CE. These include separation by ion exchange (i.e. high-performance anion exchange), normal phase HPLC, and size exclusion chromatography.

Labeled glycans can also be detected using SDS-PAGE and mass spectrometry. The dye linkage is stable to both of these methods. Mass spectrometry can be performed with either an electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI) ion source. See References for more information.

Troubleshooting Guide

Estimating sample size	The glycan content of an unknown sample may be estimated. The average glycosylation of proteins is 2-5% with an average glycan molecular weight of 1,000 Daltons. A 1 mg sample of glycoprotein contains ~ 50 µg of glycans.
Poor dye incorporation	Improper temperature control during incubation. Ensure that samples remain at 65 °C throughout the entire incubation period by preheating heat blocks. Do not over or under heat. Dye incorporation declines sharply at incubation temperatures below 65 °C. Higher temperatures will not increase dye incorporation, but will contribute to desialylation.
	Incomplete solubilization. Glycans must be completely solubilized for maximum labeling efficiency. Ensure thorough mixing at each stage of the procedure. Vortexing for 30 minutes during the incubation period may be necessary (see Procedure, step 6).
Contamination	Sample may contain contaminants that interfere with dye incorporation. Ensure that glycans have been properly purified prior to labeling.
	Degraded Labeling Solution. The Labeling Solution must be freshly prepared immediately prior to use. The Labeling Solution begins to degrade after one hour.
Lower than expected concentration of glycans	Loss or absence of free reducing sugar. The aldehyde group on the free reducing sugars must be available for 2-AA conjugation. Glycans that have been conjugated at their reducing termini (glycolipids, previously labeled glycans, glycopeptides) leave no open site for conjugation to occur.
	Loss of glycans or incomplete binding of glycans to membrane during clean-up process. Refer to the technical bulletin provided with the GlycoProfile Glycan Clean-up Cartridges (Product Code G 8169) for troubleshooting guide and recommended solutions.
Desialylation of glycans	Exposure to acidic conditions or heating at temperatures greater than 65 °C may result in desialylation.
High background, non-glycan fluorescence	This may indicate the presence of an aldehyde-bearing contaminant that readily reacts with 2-AA dye. Ensure adequate clean-up procedure prior to labeling.

Related Products
Enzymatic Deglycosylation Kit (Product Code EDEGLY)
PNGase F (Product Code P 7367)
O-Glycosidase (Product Code G 1163)

References:

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3. Delaney, J., and Vouros, P., Liquid chromatography ion trap mass spectrometric analysis of oligosaccharides using permethylated derivatives, Rapid Commun. Mass Spectrom., **15(5)**, 325-334 (2001).
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6. Hardy, M.R., Glycan labeling with the fluorophores 2-aminobenzamide and anthranilic acid, Techniques in Glycobiology, Dekker, Inc. (New York, NY: 1997).
7. Townsend, R., *et al.*, Multimode high-performance liquid chromatography of fluorescently labeled oligosaccharides from glycoproteins, Anal. Biochem., **239**, 200-207 (1996).
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