KORNBERG SEQNOSTICS
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2-AB Glycan Labeling Kit

Cat No: KS-804

Introduction:

Convenient fluorescent labeling of glycans with 2-AB (2-aminobenzamide) by reductive amination. Any purified glycan or glycan pool with a free reducing end may be labeled.

- Labeling efficiency typically >85%
- Sufficient Labeling Reagent in each reaction for up to 50 nmols of glycan (up to 48 individual samples).
- Labeling components may be stored and re-used
- Useful for profiling and quantitation

Reductive Amination Reaction

The labeling reaction involves a 2-step process
1. Schiff's Base Formation - requires a glycan with a free reducing sugar, which is in equilibrium between the ring closed (cyclic) and ring open (acyclic) forms. The primary amine group of the dye performs a nucleophilic attack on the carbonyl carbon of the acyclic reducing sugar residue to form a partially stable Schiff's base.

2. Reduction of the Schiff's Base - the Schiff's base imine group is chemically reduced by cyanoborohydride to give a stable, labeled glycan.

Materials Provided:

1. 2-AB Solution – 1 vial
2. Reductant Solution – 1 vial

Materials Provided by the End-User:

1. Kornberg S Cartridges (Cat No KNBS-9726),
2. Water, HPLC grade
3. Acetonitrile, HPLC grade
4. Heating block, oven or similar dry heater set at 65°C
5. Centrifugal evaporator (e.g., Savant, Heto or similar)
6. Reaction vials (e.g., polypropylene microcentrifuge vials)

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Sample Preparation:

The amount of sample should be in the range of 100 picomoles to 50 nanomoles for a glycan pool obtained from a typical glycoprotein. With a single pure glycan, as little as 5 picomoles may be labeled. Dry the aqueous samples in a centrifugal evaporator.
NOTE: Lyophilization may be used with caution. Specifically, ensure that the sample dries to a small, compact mass at the very bottom of the tube.

Reagent Preparation:

Allow the 2-AB Solution and Reductant Solution vials to come to room temperature in the sealed desiccant bag before removing them. Before opening each vial, flick it or gently tap it on a flat surface to dislodge any liquid adhering to the underside of the cap and ensure that the contents collect at the bottom.

In a separate vial, prepare a Labeling Reagent master mixture:
Determine the number of samples to be labeled. For each sample to be processed, add 3 ul of 2-AB Solution and 3 ul of Reductant Solution.

Cap tightly and vortex on high for 10 seconds to mix; briefly spin down in a centrifuge. Tightly cap the 2-AB Solution and Reductant Solution vials, return to the desiccant-containing bag and store at -20°C.

NOTE:
1. 2-AB Labeling Reagent should be prepared no more than one hour before use.
2. 2-AB Labeling Reagent components are hazardous.
3. Both the 2-AB Solution and Reductant Solution are hygroscopic; minimize exposure to air and protect from exposure to light. The individual reagents may be resealed, repackaged with the desiccant in the resealable bag, and frozen (-20^{0}C) for storage up to 6 months; return to RT before opening for use to minimize condensation.

Assay Procedure:

Labeling

Add 5 ul of Labeling Reagent to each dried glycan sample, cap the microtube, mix thoroughly, and gently tap or centrifuge at low speed to ensure the contents are at the bottom of the vial.
Place the reaction vials in a heating block, sand tray or dry oven set at 65°C. Incubate for 3 hours.
NOTE:
The incubation should be performed in a dry environment
In most cases, the incubation time may be shortened to 1 hour or extended to 4 hours without significantly changing the outcome.

After incubation, centrifuge each reaction tube briefly to incorporate any liquid that may have condensed on the top and sides.

Post-Labeling Cleanup

Sample cleanup to remove excess dye and other labeling reagents is necessary for certain applications, e.g., subsequent analysis by liquid chromatography. Cleanup can be achieved using Kornberg's S Cartridges Cat No. KNBS-9726.

Analysis of Glycans Labeled with 2-AB
Use standard techniques, such as Liquid Chromatography (LC), Mass Spectrometry (MS), or a combination of the two, to analyze the aqueous eluate containing eluted, labeled N-glycans.

Summary of Assay Procedure:

1. Prior to labeling, glycan samples should be purified to remove protein, peptides, salts, detergents and any other contamination that could interfere with the Labeling Procedure.
Examples of glycan isolation protocols include:
• Cold ethanol precipitation
• Molecular weight cut off filtration
• Solid phase extraction (e.g., HILIC, normal phase, reversed phase)

2. Each sample is placed in a reaction vial and dried.

3. Labeling Reagent is prepared fresh by mixing components supplied in the kit.

4. Labeling Reagent is added and the samples incubated at 65C for 1-4 hours.

5. Excess Labeling Reagent may be removed from the samples using the Kornberg's S Cartridges