

2-AB Labeling Kits

A492565-02

Application

For labeling of free glycans with 2-aminobenzamide acid (2-AB).

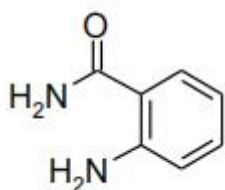
Description

The kit contains reagents for the conjugation of dye to the free reducing end of the glycan by a reductive amination reaction.

Dye Properties

Mass = 136.15

Fluorescence, $\text{ex} = 250 \text{ nm}$, $\text{em} = 428 \text{ nm}$.



Number of Samples

Up to 15 separate analytical samples per set of labeling reagents (up to 30 samples in total for the kit)

Amount of Sample

From 25 pmol up to 25 nmol glycans per sample.

Suitable Samples

Any purified glycans with free reducing termini can be labeled.

Structural Integrity

No detectable (< 2 mole per cent) loss of sialic acid, fucose, sulfate, or phosphate.

Labeling Selectivity

Essentially stoichiometric labeling.

Storage:

Store at room temperature in the dark. Protect from sources of heat, light, and moisture. The reagents are stable for at least two years from the manufacture date.

Shipping:

The product can be shipped at ambient temperature.

Handling:

Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates. Use powder-free gloves for all sample handling procedures and avoid contamination with environmental carbohydrate. All steps involving labeling reagents must be performed in a dry environment with dry glassware and plasticware. Once individual vials of reagents are opened, their contents should be used immediately and excess then discarded according to local safety rules.

Safety:

For research use only. Not for human or drug use

Please read the Safety Data Sheets (SDS's) for all chemicals used. All processes involving labeling reagents should be performed using appropriate personal safety protection - eyeglasses, chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard.

Kit Contents

Each kit contains two labelling reaction sets. Each labeling reaction set consists of one vial of each of the following:

A492565-02A	2-AB Dye (2-aminobenzamide)	7.5 mg
A492565-02B	2PB reductant (2-picoline borane)	16.5 mg
A492565-02C	30% acetic acid in DMSO	500 µL

Additional Reagents and Equipment Required

- Milli Q water or similar
- Heating block, oven, or similar dry heater (a water bath cannot be used) set at 65°C
- Centrifugal evaporator (e.g. Savant, Heto, or similar)
- Reaction vials (e.g. polypropylene microcentrifuge vials)
- Note: Further reagents are required if doing the optional post-labeling sample cleanup (see Section on Sample Cleanup)

Time Line for Labeling

The2-AB labeling procedure takes 2 hours with just 1 hour for the actual labelling incubation.

Procedure	Time	Elapsed Time (hours)
Transfer samples to reaction tube and dry	30 min	0.5
Add water to samples	15 min	0.75
Make up and add labeling reagent	15 min	1
Incubate samples with reagent	1 hour	2

Labeling Method

1 Purify the glycans

If necessary, remove non-carbohydrate contaminants from the samples

2 Transfer sample to reaction vial

The kit is designed to label up to 25 nmols of glycans per reaction. With a single pure glycan as little as 5 pmols per reaction can be labeled and detected in subsequent HPLC analysis. Suitable reaction vials include small polypropylene microcentrifuge tubes and tubes for PCR work.

3 Dry the samples and redissolve in 10 μ L of water

Dry down the samples if the volume of the sample exceeds 10 μ L.

If the samples need to be dried down then this should be done using a centrifugal evaporator. If this is not possible then freeze drying (lyophilization) can be used with

caution (in particular, ensure that the sample dries to a small, compact mass at the very bottom of the vial). Do not subject samples to high temperatures ($>28^{\circ}\text{C}$) or extremes of pH as these conditions will result in acid catalysed loss of sialic acids (high temperatures, low pH) or epimerization of the glycan reducing terminus (at high pH). Once the samples are dry then redissolve the glycans in 10 μL of water.

4 Prepare the labelling reagent

Add 150 μL of kit component A492565-02C (30% acetic acid in DMSO) to a vial of dye and mix by pipette action until the dye is dissolved. Sometimes heat ($30\text{--}60^{\circ}\text{C}$) is required to help dissolve the dye.

Transfer the 150 μL of dissolved dye solution to a vial of reductant (A492565-02B) and mix by pipette action until the reductant is dissolved. Sometimes heat ($30\text{--}60^{\circ}\text{C}$) is required to help dissolve the reductant.

5 Add labeling reagent to samples

Add 10 μL of labeling reagent to each glycan sample, cap the microtube, mix thoroughly, and then gently tap to ensure the labeling solution is at the bottom of the vial.

6 Incubate

Place the reaction vials in a heating block, sand tray, or dry oven set at 65°C and incubate for 1 hour.

The samples must be completely dissolved in the labeling solution for efficient labeling. To encourage complete dissolution the samples can be vortexed 10 minutes after the start of the 65°C incubation then the incubation continued.

7 Centrifuge and cool

After the incubation period remove the samples, centrifuge the microtubes briefly, and then allow them to cool completely to room temperature.

The Reductive Amination Reaction

The labeling reaction involves a two step process (see Figure 1):

1. Schiff's base formation.

This requires a glycan with a free reducing terminus which is equilibrium between the ring closed (cyclic) and ring open (acyclic) forms. The primary amino group of the dye performs a nucleophilic attack on the carbonyl carbon of the acyclic reducing terminal 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 to give a stable labeled glycan.

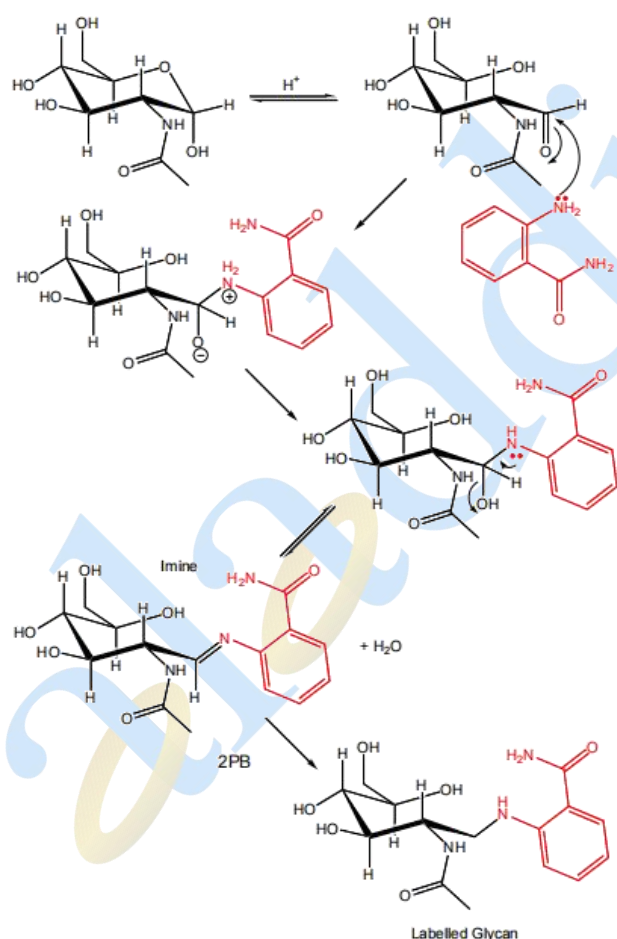


Figure 1: Labeling of a glycan with 2-aminobenzamide acid (2-AB) by reductive

amination.

Appendix 1: Troubleshooting Guide

The glycan sample to be labeled, whether a purified glycan or a glycan mixture, must contain a free reducing terminus, be particle and salt-free, and be presented in a volatile solvent system (preferably pure water).

The following may interfere with the labeling reaction and must be removed from the glycan samples prior to labeling:

- Non-volatile solvents
- Non-volatile salts, in particular transition metal ions
- Detergents
- Dyes and stains such as Coomassie Blue

The 2-AB Labeling Kits labeling protocol is an efficient, robust method. If problems do arise they can normally be corrected without difficulty. The following is a guide to the most likely problems, possible causes, and solutions.

Poor Incorporation of Dye / Low Labeling Yield

Water was not added to the glycans prior to adding the labelling reagent

Please ensure that 10 μ L of water is added to the glycans during the labelling step. The water can be used to solubilise the glycans prior to adding the labelling reagent or added after the labelling reagent. Water is necessary in order to maximise the labelling efficiency.

The labeling temperature was incorrect.

Please ensure that the oven or heating block is equilibrated to the incubation temperature and that the reaction tube is subjected to this temperature for the entire labeling period.

The sample was incompletely solubilised.

The glycans must be completely dissolved in the labeling mixture for maximum labeling efficiency. Please ensure that the sample is thoroughly mixed with the labeling reagent prior to the incubation and, as a precaution, carefully mix the samples 15 minutes after the start of the incubation.

The sample contained contaminants that interfered with the labeling.

Please ensure that the glycans are adequately purified before labeling (see protocol step 1).

The labeling solution was inactive.

Please make up the labeling solution immediately before use - the reagents will lose activity within a few hours of mixing.

There was less starting glycan than was originally estimated.

The glycans did not contain a free reducing terminus.

The 2-AB dye conjugates to the glycan via the aldehyde group of the free reducing terminus. Alditols and glycans already conjugated via their reducing terminus (e.g. glycopeptides, glycolipids, and previously labeled glycans) do not contain a free reducing terminus and so cannot conjugate to the dye.

The glycans were lost during the post-labeling cleanup.

Please ensure that the removal of excess labeling reagents is performed as specified in the cleanup protocol and that the wash reagents are correctly made.

The Labeled Samples Contain Fluorescent Non-Carbohydrate Material

The original glycans contained aldehyde-bearing contaminants.

Please ensure that the glycans are adequately purified before labeling.

The post-labeling cleanup step did not work correctly.

Please ensure that the removal of excess labeling reagents is performed as specified in the post-labeling cleanup protocol and that the wash reagents are correctly made.

Selective Loss of Smaller Glycans

The cleanup cartridge was not primed correctly.

Please ensure the cartridge is primed correctly and that the cartridge bed is still wet with acetonitrile when the sample is applied to the disc.

Incorrect wash reagents were used during the post-labeling cleanup.

Please ensure that the wash reagents are correctly prepared.

Selective Loss of Larger Glycans

The sample was incompletely solubilised.

The glycans must be completely dissolved in the labeling mixture for maximum labeling efficiency. Larger glycans tend to be less soluble in the labeling mixture than small sugars. Please ensure that the sample is thoroughly mixed with the labeling reagent prior to the incubation and, as a precaution, carefully mix the samples 15 minutes after the start of the incubation.

Desialylation of the Glycans

The sample was subjected to acidic conditions in aqueous solutions at elevated temperatures

Avoid prolonged periods of exposure of sialylated glycan samples in aqueous solutions to conditions of low pH and elevated temperatures. Keep the incubation time of the labelling reaction to 1h as desialylation increases with 2 or 3h incubation times.

The samples were not cleaned up correctly after labeling

Make sure that samples undergo the post-labeling cleanup immediately after the reductive amination reaction and that the post-labeling drying and cleanup procedure is conducted reasonably quickly.



Labeled samples that have not undergone drying and subsequent cleanup will be prone to acid catalyzed desialylation.

