



## LudgerTag 2-AB Labeling Kit

### Catalog number

LT-KAB-A2

### Application:

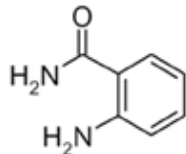
The 2-AB labeling kits are designed for the fluorophore or chromophore labeling of glycans with a free reducing terminus. Labeled glycans may be detected by either high-sensitivity fluorescence detection or monitoring of UV-absorbance during various chromatographic and structure sequence analyses. These include chromatography on QA-Bio HPLC columns and sequencing using exoglycosidases.

### Dye properties:

2-Aminobenzamide acid

Mass = 136.15

Flourescence: excitation = 320-330 nm  
emission = 420 nm



### Number of samples

Typically, up to 15 separate analytical samples per set of labeling reactions. Two sets are included per order.

### Amount of sample:

From 25 pmol up to 25 nmol glycans

### Sample type:

Any purified glycans with free reducing termini can be labeled.

### Labelling efficiency:

Typically > 85% (dependant on sample)  
Essentially stoichiometric labelling.

### Storage:

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

### Structural integrity:

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

### Kit contents:

- 2-AB Dye (2-Aminobenzamide acid)
- DMSO
- Acetic acid
- Sodium cyanoborohydride

### Additional reagents & equipment required:

- Heating block, oven, or similar dry heater (a water bath should not be used) set at 65°C
- Centrifugal evaporator (e.g. Savant, Heto, or similar)
- Reaction vials (polypropylene microcentrifuge tubes)
- Note: Further reagents are required if doing the post-labeling sample cleanup- LudgerClean S cartridges recommended (LC-S-01)

LT-KAB-A2 2-AB Labeling Kit

Specifications - Protocol

**Protocol outline:**

<i>Procedure</i>	<i>Time</i>
Transfer & dry samples	30 min
Prepare & add labeling reagent	15 min
Incubate samples	3 hrs
Post-labeling cleanup	1 hour
elapsed time:	4 hrs 45 minutes

**Sample Preparation:**

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

Our LudgerClean E Cartridges (LC-EB10-A6) can be used for desalting and removal of detergents.

The standard sample preparation protocol is as follows:

**1 Purify the glycans**

If necessary, remove non-carbohydrate contaminants from the samples

**2 Transfer sample to reaction vial**

The amount of sample should be in the range 100 picomoles - 50 nanomoles for a glycan pool obtained from a typical glycoprotein. With a single pure glycan as little as 5 picomoles 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**

Ideally, samples should be dried 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).

## Preparation of Labeling Reagent

### 4 Prepare a DMSO-acetic acid mixture

Add 150 µl glacial Acetic Acid to the vial of DMSO and mix by pipette action.

*Open the ampoules by carefully tapping or flicking to dislodge any contents in the upper half, then carefully break open the ampoule.*

*If the DMSO is frozen then gently warm up the vial (before opening) in an oven or heating block to between 30° C and 65° C.*

### 5 Add the dye

Add 100 µl of the DMSO-acetic acid mixture to a vial of LudgerTag 2-AB Dye (2-Aminobenzamide acid)

Dye and mix until the dye is dissolved.

### 6 Add the reductant

Add the dissolved dye to a vial of Sodium Cyanoborohydride (reductant) and mix by pipette action until the reductant is completely dissolved to make the final labeling reagent.

*If the reductant is difficult to dissolve then gently warm the vial for up to four minutes in the 65° C incubation oven or stand on a heating block at this temperature then mix by pipette action. If undissolved reductant is still visible add 10 µl pure water to the vial and mix.*

*Protect the labeling reagent from exposure to moisture and use within 60 minutes.*

### 7 Add labeling reagent to samples

Add 5 µl of labeling reagent to each dried glycan sample, cap the microtube, mix thoroughly, and then gently tap to ensure the labeling solution is at the bottom of the vial.

### 8 Incubate

Place the reaction vials in a heating block, sand tray, or dry oven set at 65° C and incubate for 3 hours. *The incubation must be performed in a dry environment. Use an oven or dry block - please do not use a water bath.*

*The samples must be completely dissolved in the labeling solution for efficient labeling. To encourage complete dissolution the samples can be vortexed 30 minutes after the start of the 65° C incubation, then continuing the incubation.*

*In most cases, the incubation time can be shortened to 2 hours or extended up to 4 hours without significantly changing the outcome of the labeling reaction.*

### 9 Centrifuge and cool

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

**QA-Bio S Cartridge Post-Labeling Sample Cleanup**

Post-labeling sample cleanup (to remove excess dye and other labeling reagents) is necessary for certain applications - e.g. subsequent analysis by HPLC. Such cleanup can be achieved using LudgerClean S cartridges (Cat #LC-S-01) using the standard protocol included with the kit.

Post-labeling sample cleanup is not necessary for applications where the excess labeling reagents do not interfere with subsequent sample analysis. These include carbohydrate electrophoresis where free dye runs away from the labeled glycans.

**Analysis of 2-AB Labeled Glycans**

2-AB labeled glycans may be studied by a number of different analytical methods including HPLC, gel electrophoresis, and mass spectrometry.

**HPLC Analysis**

2-AB labeled glycan mixtures may be separated and analyzed by a variety of HPLC (high pressure liquid chromatography) methods. The QA-Bio columns include the following:

<b>Types of Analyses</b>	<b>Column</b>	<b>Cat. No.</b>
Separation by charge	LudgerSep C2 Anion Exchange	LS-C2-4.6x50
Separation by hydrophilicity (size)	LudgerSep N1 Amide-80	LS-N1.4.6x250

**Enzymatic Analysis**

High purity, sequencing grade enzymes (e.g. exoglycosidases) suitable for structural analysis of both N- and O-linked glycans are available from QA-Bio. We have compiled a set of these enzymes into our CarboSequencing kit (Catalog # KE-SQ01), designed for the analysis of mammalian glycoproteins.

**Mass Spectrometry and Electrophoresis**

QA-Bio labeled glycans may also be analyzed by mass spectrometry, electrophoresis, and various types of spectroscopy. Please call us for advice on the analysis conditions most suitable for your intended use.

## References

Bigge, J.C.; Patel, T.P; Bruce, J.A.; Goulding, P.N.; Charles, S.M; Parekh, R.B. (1995)  
'Non-selective and efficient fluorescent labeling of glycans using 2-aminobenzamide and anthranilic acid'. Analytical Biochemistry **230**: 229-238

Guile, G.R.; Rudd, P.M.; Wing, D.R.; Prime, S.B.; Dwek, R.A. (1996)  
'A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles'. Analytical Biochemistry **240**: 210-226

Townsend, R.R.; Lipniunas, P.H.; Bigge, C.; Ventom, A.; Parekh, R. (1996)  
'Multimode high-performance liquid chromatography of fluorescently labeled oligosaccharides from glycoproteins'. Analytical Biochemistry **239**: 200-207

Hardy, M.R. (1997)  
'Glycan labeling with the fluorophores 2-aminobenzamide and anthranilic acid' in 'Techniques in Glycobiology', edited by Townsend, R.R and Hotchkiss, A.T.. Marcel Dekker Inc, New York .

## Warranty

QA-Bio warrants that the above product conforms to the specification described herein. Should the product fail for reasons other than through misuse, QA-Bio will, at its option, replace the product free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warranties, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose. QA-Bio shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research use only. merchantability or fitness for any particular purpose. QA-Bio shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research use only.

*updated June 7, 2007*



# LudgerTag Glycan Labeling Kits

2-AA Labeling Kit LT-KAB-A2  
2-AB Labeling Kit LT-KAA-A2



## 1. Purify the Glycan

Before labeling remove any contaminants (such as salts and detergents) that could interfere with the labeling reaction. In most cases this can be done using one of our E Cartridges for glycan purification (C-E001).



## 2. Aliquot Sample

Aliquot a suitable amount of sample (typically it should contain from 1 pmol to 10 nmol of glycan) into a microcentrifuge tube.



## 3. Dry the Glycan

Dry the sample. This is best done using centrifugal evaporation as this gives minimal sample loss and the dried glycan ends up at the bottom of the tube.



## 4. Check the Dried Sample

Inspect the sample to make sure that it looks clean. There should be no visible contaminants or discoloration. In most cases, unless you have incredibly good eyesight, you won't be able to see the sample either.



## 5. Select the Labeling Kit

Select the LudgerTag kit that you will be using. Let the unopened kit equilibrate to ambient temperature if it has been stored at a different temperature. The kit consists of a Ludger Cassette which holds several reagent ampoules each of which is housed in a protective container.



## 6. Remove One Set of Ampoules

Remove one set of labeling reagent ampoules (containing acid, solvent, dye, and reductant) from the Cassette. To take the ampoules out of their protective containers use forceps to first remove the topmost foam shock absorber then gently pull out the glass ampoule.



### 7. Open the Ampoules

To open a glass ampoule hold the body and top gently but firmly then carefully snap it open at the coloured break ring. Make sure you are wearing appropriate personal protection (suitable gloves, safety glasses, lab-coat) and that you snap in the direction away from your body. Be careful of the snapped edges which may be sharp.



### 8. Add Acetic Acid to DMSO

Add a prescribed volume of acetic acid to the ampoule of DMSO then mix by pipette action.



### 9. Add DMSO/acetic acid mix to the Dye

Add 100  $\mu$ l of the DMSO-Acetic acid mixture to the dye. Mix until dye is dissolved.



### 10. Add Dye Mixture to Reductant

Add the acid-DMSO-dye mixture to the vial of reductant. For most kits the reductant is sodium cyanoborohydride.



### 11. Add Labeling Reagent to Glycan

Add a prescribed amount (typically 5  $\mu$ l) of the acid-DMSO-dye-reductant labeling mixture to each dried glycan sample. Encourage the dissolution of the glycan by gently tapping the closed sample tube on the lab bench. If necessary, centrifuge to ensure that the labeling mixture is at the very bottom of the tubes.



### 12. Incubate

Incubation conditions are typically 65 C for 3 hours (depending on the particular labeling kit). Incubation should be done in clean, dry conditions in an oven or heating block. Do not use a heated fluid (e.g. oil or water bath).



### 13. Post-Labeling Purification

In most cases the excess labeling reagents must be removed before further work. This can be done using one of our S cartridges (LC-S-01).

### 14. Store the Labeled Glycan

Place the sample tubes in a moisture-proof container (e.g. plastic food-type container) and store at -20 C or lower away from light. Samples can be stored either as aqueous solutions or in dried form.