Ludger™

LudgerTag™ 2-AP [2-Aminopyridine] Glycan Labeling Kit

Instruction Guide

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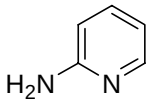
LudgerTag 2-AP Glycan Labeling Kit Specifications

Application For labeling of glycans with fluorescent 2-AP [2-aminopyridine] dye.

Dye Properties Mass = 94

Fluorescence: λ_{ex} = 295 nm, λ_{em} = 352 nm



Description The kit contains reagents for the conjugation of 2-AP dye to the free reducing end of glycans by a reductive amination reaction.

Number of Samples Typically, up to 15 separate analytical samples per set of labeling reagents.

Amount of Sample Typically, from 1 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 efficiency Typically > 85 % .

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 as supplied.

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: Please read the Material Safety Data Sheets (MSDS'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

For research use only. Not for human or drug use

Kit Contents

Each labeling reaction set consists of one vial of each of the following :

Cat. #	Item	Quantity
LT-AP-01	2-AP [2-aminopyridine] Dye	20 mg
LT-DMSO-01	DMSO [Dimethyl sulfoxide]	350 µl
LT-ACETIC-01	Acetic acid	200 µl
LT-CYANOB-02	Sodium cyanoborohydride (Reductant)	12 mg

Additional Reagents and Equipment Required

- Pure water
- Heating block, oven, or similar dry heater (a water bath cannot be used) set at 80 ^{O}C
- Centrifugal evaporator (e.g. Savant, Heto, or similar)
- Reaction vials (e.g. polypropylene microcentrifuge vials)

Additional Materials Needed For Post-Labeling Sample Cleanup

There are a number of alternative post-labeling cleanup procedures that can be used.

Post-Labeling Cleanup Protocol A: Cation Exchange Cleanup

- AG 50W-X8 cation exchange resin (Bio-Rad, Cat # 143-1451) or
  Dowex 50Wx8 (200-400 mesh) (Supelco, Cat # 50X8200-100G)
- 1 M ammonium acetate buffer (pH 6.8)
- 20 mM ammonium acetate buffer (pH 6.8)
- 20 mM ammonium acetate buffer (pH 8.5)
- Small chromatography column (e.g. Supelco polypropylene filtration tubes, 3 ml Cat # 57024 or 1 ml Cat # 57023)

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 (acylic) 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.

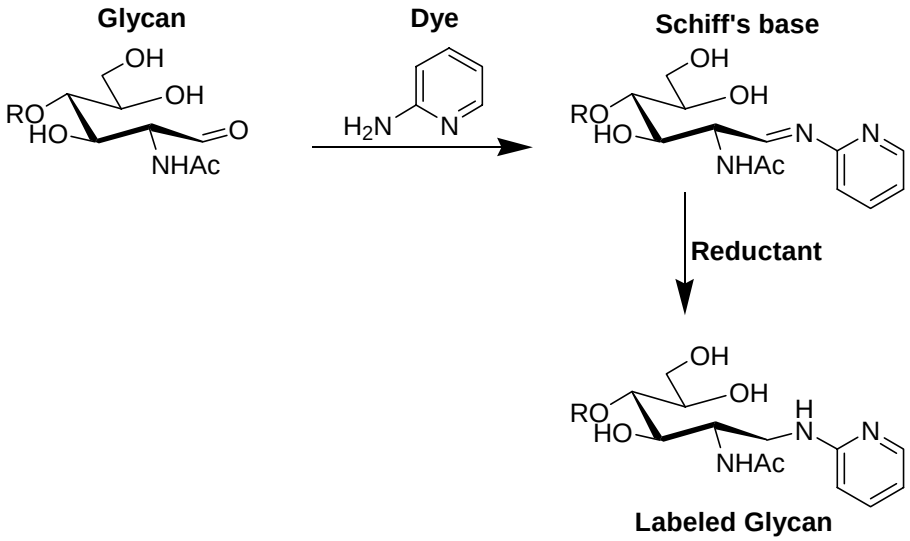


Figure 1: Labeling of a glycan with 2-aminopyridine (2-AP) by reductive amination.

Outline of Labeling Protocol

The LudgerTag™ 2-AP glycan labeling kit is designed for labeling with 2-AP [2-aminopyridine] of glycans with a free reducing terminus. 2-AP labeled glycans may be followed by high-sensitivity fluorescence detection during various chromatographic and structure sequence analyses. These include chromatography on LudgerSep™ HPLC columns and sequencing using exoglycosidases (See refs 1-5, 7-8).
The 2-AP labeling procedure used here follows the protocol given in reference 8. The outline of the procedure is as follows :

- **Prepare the glycans.**

  Prepare the glycan samples by removing contaminants such as salts and detergents which could interfere with the labeling procedure. The glycans must have free reducing termini. The glycans of glycoconjugates such as glycoproteins and glycolipids may be released by either chemical means (e.g. hydrazinolysis) or enzymatic treatment (e.g. using endoglycosidases such as PNGase F).

- **Dry the glycans**

  Place the samples in reaction vials and dry down.

- **Perform the labeling reaction**

  Use one of two alternative labeling protocols:

  Labeling Protocol A: One step reaction

  This is a simpler and faster procedure than Protocol B and is suitable for most routine analyses.

  - Prepare labeling reagent containing dye and reductant
  - Add labeling reagent to the glycan sample
  - Incubate

  Labeling Protocol B: Two step reaction

  This is a longer and complicated procedure than Protocol A but is reported to give better labeling efficiencies.

  - Add dye reagent to the glycan sample
  - Incubate
  - Add reductant reagent to the glycan sample
  - Incubate

- **Post-labeling cleanup**

  After incubation, remove the excess labeling reagents using a straightforward cleanup procedure.

- **Store or analyse the labeled glycans**

  The labeled glycans are now ready for analysis.

Time Line for Labeling

The LudgerTag 2-AP labeling procedure including the optional post-labeling sample cleanup typically takes around 4 - 5 hours :

Procedure	Time	Elapsed Time (hours)
Transfer samples to reaction tube and dry	0.5 - 1 hour	1
Make up and add labeling reagent	15 min	1.25
Incubate samples	1 hour	2.25
Post-labeling cleanup	1 - 2 hour	4.25

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 reductive amination reaction and must be removed from the glycan samples prior to 2-AP labeling:

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

A range of LudgerClean kits for cleaning glycan samples prior to 2-AP labeling is available from Ludger. These are summarized in the LudgerClean Glycan Cleanup Guide [ref 6]. Please contact us for advice regarding your particular application.

The standard sample preparation protocol is as follows :

1 Purify the glycans

If necessary, remove non-carbohydrate contaminants from the samples using one of the strategies outlined in the Glycan Cleanup Guide [ref 6].

2 Transfer the samples to reaction vials

The amount of sample should be in the range 10 picomoles - 50 nanomoles for a glycan pool obtained from a typical glycoprotein. With a single pure glycan as little as 1 picomole 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).

Do not subject samples to high temperatures (> 30 ^{o}C) or extremes of pH as these conditions will result in acid catalyzed loss of sialic acids (at high temperatures and low pH) or epimerization of the glycan reducing terminus (at high pH).

Go to either:

- Labeling Protocol A: One Step Reaction (steps 4 - 10) or
- Labeling Protocol B: Two Step Reaction (steps 11 - 20).

Labeling Protocol A: One Step Reaction

This labeling protocol uses a one step reaction in which both the dye (2-aminopyridine) and reductant (sodium cyanoborohydride) are added together. This is a simpler and faster procedure than the alternative two-step reaction of Protocol B (see steps 11 - 20).

4 Prepare a DMSO-acetic acid mixture

Add 62 µl glacial **Acetic Acid** to the vial of **DMSO** and mix by pipette action.

The Catalog #s for the acetic acid and DMSO are LT-ACETIC-01 and LT-DMSO-01 respectively.
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 set at between 30^{O}C and 80^{O}C.

5 Add the 2-AP dye

Add 400 µl of the DMSO-acetic acid mixture to a vial of **2-AP Dye** and mix until the dye is dissolved.

The Cat. # for the dye is LT-AP-01.

6 Add the reductant

Make the final **labeling reagent** by adding the dissolved dye to a vial of **Sodium Cyanoborohydride** (reductant). Mix by pipette action until the reductant is completely dissolved.

The Cat. # for the sodium cyanoborohydride reductant is LT-CYANOB-02.
If the reductant is difficult to dissolve then gently warm the vial for up to four minutes in an incubation oven between 65-80^{O}C or stand on a heating block at this temperature then mix by pipette action.
Protect the labeling reagent from exposure to moisture and use within 60 minutes.

7 Add labeling reagent to samples

Add 20 µl of labeling reagent to each dried glycan sample, cap the microtube, mix thoroughly, 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 80 ^{o}C and incubate for 30 minutes.

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 5 minutes after the start of the 80 ^{o}C incubation then

the incubation continued.

An alternative incubtation regime that gives equivalent results is 70 ^{o}C for 2 hours .

9 Centrifuge and cool

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

10 Stop the reaction by drying

The labeling reaction is stopped by drying the samples. This can be done either by freeze-drying or centrifugal evaporation.

Labeling Protocol B: Two Step Reaction

This follows the traditional two-step 2-AP labeling protocol:

- **Step One** (Protocol steps 11-14): This involves incubating the dye (2-aminopyridine) with the glycan to form a Schiff's base.
- **Step Two** (Protocol steps 15-20): The Schiff's base is stabilized by chemical reduction with sodium cyanoborohydride.

This procedure is more complex and takes longer than Protocol A but is reported to give greater labeling efficiencies.

11 Prepare a DMSO-acetic acid mixture

Add 62 µl glacial **Acetic Acid** to the vial of **DMSO** and mix by pipette action.

The Catalog #'s for the acetic acid and DMSO are LT-ACETIC-01 and LT-DMSO-01 respectively.
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 set at between 30^{O}C and 80^{O}C.

12 Prepare the 2-AP dye solution

Add 200 µl of the DMSO-acetic acid mixture to a vial of **2-AP Dye** and mix until the dye is dissolved.

The Cat. # for the dye is LT-AP-01.
Keep the remaining DMSO-acetic acid solution by placing the vial in its protective plastic container and sealing the lid tightly. Store at room temperature until required (see step ??).

13 Add the dye solution to samples

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

14 1st incubation

Place the reaction vials in a heating block, sand tray, or dry oven set at 80 ^{o}C and incubate for 1 hour.

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 5 minutes after the start of the 80 ^{o}C incubation then the incubation continued.

15 Prepare the reductant solution

Make the final **reductant solution** by adding 200 µl of the DMSO-acetic acid mixture to a vial of **Sodium Cyanoborohydride** (reductant). Mix by pipette action until the reductant is completely dissolved.

The Cat. # for the sodium cyanoborohydride reductant is LT-CYANOB-02.
If the reductant is difficult to dissolve then gently warm the vial for up to four minutes in an incubation oven between 65-80^{O}C or stand on a heating block at this temperature then mix by pipette action.
Protect the reductant solution from exposure to moisture and use within 60 minutes.

16 Centrifuge and cool

Remove the samples from the incubator, centrifuge the microtubes briefly, then allow them to cool completely to room temperature.

17 Add the reductant solution to samples

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

18 2nd incubation

Place the reaction vials in the incubator at 80 ^{o}C and incubate for a further 30 minutes.

The incubation must be performed in a dry environment. Use an oven or dry block - please do not use a water bath.

19 Centrifuge and cool

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

20 Stop the reaction by drying

The labeling reaction is stopped by drying the samples. This can be done either by freeze-drying or centrifugal evaporation.

Post-Labeling Sample Cleanup

There are a number of different methods described in the literature for post-2-AP labeling sample cleanup (to remove excess labeling reagents). These include gel filtration, liquid-liquid extraction, and solid-phase extraction using ion-exchange resins.

Efficient methods for removal of excess 2-AP include the following:

- Dissolution of the sample in saturated sodium bicarbonate and removal of free 2-aminopyridine by liquid-liquid extraction with benzene [ref 11].
- Extraction of excess 2-AP with phenol / chloroform [ref 12].

The method recommended below (Post-Labeling Cleanup Protocol A) is suitable for most studies and does not involve the use of organic solvents.

Post-Labeling Cleanup Protocol A: Cation Exchange Cleanup

This is based on the cleanup protocol outlined in reference 10 and uses a cation exchange resin in the NH_4^+ form to selectively bind excess 2-AP dye. This method is fast, efficient, inexpensive, uses readily available materials, and the reagents are more acceptable than those used in other cleanup procedures.

21 Prepare the NH_4^+ form cation exchange resin

Clean the resin (either Bio-Rad AG 50W-X8 or Dowex 50W-X8) as follows:

- Sedimentation wash the resin by suspending it in water (around 10 times more water than the settled bed volume of the resin), allow to settle, then drain off the supernatant. The latter may be slightly coloured and contain suspended fine particles which need to be removed.
- Repeat the water sedimentation wash several times until all the washes are totally clear (normally at least 10 washes are required).
- Sedimention wash with 1 M ammonium acetate pH 6.8 (5 times).
- Sedimentation wash with water (10 times).
- Sedimention wash with 20 mM ammonium acetate pH 6.8 (5 times).
- Store the resin at 4 ^oC until required .

11 Prepare the cleanup cartridge

For each sample prepare one cleanup cartridge as follows:

- Add a total of 0.2 ml settled bed volume of $NH4_4^+$ form cation exchange to a clean chromatography column.
- Wash with 5 x 0.5 ml aliquots of 20 mM ammonium acetate pH 6.8. Allow the fluid from the

previous wash to drain before adding the next aliquot.

22 Load the sample

- Re-constitute the dried sample in 0.2 ml water.
- Load the sample onto the cleanup cartridge.

23 Elute the labeled glycans

Elute the 2-AP labeled glycans with 5 x 1 ml of 20 mM ammonium acetate (pH 8.5).

24 Dry the sample

Dry the eluted glycans by centrifugal evaporation.

Dried samples may be stored at temperatures of –20 ^{o}C or lower.

Analysis of LudgerTag 2-AP Labeled Glycans

LudgerTag 2-AP labeled glycans may be studied by a number of different analytical methods including HPLC, electrophoresis, and mass spectrometry. These are covered in detail in reference 8 and overviewed below.

Fluorescence Detection of 2-AP Labeled Glycans

The fluorescence characteristics of 2-AP labeled glycans are summarized in reference 8. Typical settings for online fluorescence detection for HPLC are λ_{ex} = 442 nm, λ_{em} = 525 nm.

HPLC Analysis

2-AP labeled glycan mixtures may be separated and analysed by a variety of HPLC methods including chromatography on amide and reverse-phase columns [see reference 8].
LudgerSep™ HPLC columns suitable for 2-AP glycan analysis include the following :

Types of Analyses	Column	Cat. No.
Separation of charged and neutral glycans	LudgerSep C	LS-C-01
Separation using different selectivity to LudgerSep C	LudgerSep H	LS-H-01
Profile analysis of neutral and charged glycans	LudgerSep N	LS-N-01
Separation of neutral glycans	LudgerSep R	LS-R-01

The uses of these columns for glycan analysis are overviewed in Reference 4.

The LudgerSep N column is an especially powerful tool for the purification and analysis of LudgerTag labeled oligosaccharides from complex glycan mixtures. Please contact us for advice regarding your particular application.

Mass Spectral Analysis

2-AP labeled glycans are well-suited for analysis by MALDI-TOF mass spectrometry [reference 8]. To date, the matrix that has proven the most useful is dihydroxybenzoic acid at relatively high concentration (100 mg / ml). This is used with high laser energy to allow study of sialylated species that are normally difficult to analyse by MALDI-MS.
Please contact us for more information on MS analysis of 2-AP labeled glycans.

Capillary Electrophoresis (CE)

2-AP labeled glycans have been studied with success by both free zone capillary electrophoresis and MECC

using LIF excitation and detection [reference 8]. The 2-AP label confers properties on the glycans that make them particularly suitable for capillary electrophoretic analyses. These include retention of fluorescence characteristics at low pH and ionization properties compatible with acidic CE buffers.

Enzymatic Analysis

High purity, sequencing grade enzymes (e.g. exoglycosidases) suitable for structural analysis of both N- and O-linked 2-AP labeled glycans are available from a number of companies.

When selecting glycosidases be especially careful to chose those with formulations that are compatible with your particular application. For example, some enzymes and enzyme buffers have components that interfere with certain types of analysis. Please call us for guidance in chosing enzymes and reaction conditions for your work.

Warranties and liabilities

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

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

Document Revision Number

Document # ' LT-KAP-Ax-Guide', revision v1.3

References

1 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

2 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

3 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

4 LudgerSep™ High Resolution HPLC Carbohydrate Profiling Guide (Cat # LS-GUIDE-01)

5 Ludger Enzyme Selection Guide (Cat # EZ-GUIDE-01)

6 LudgerClean™ Glycan Cleanup Guide (Cat # LC-GUIDE-01)

7 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 .

8 Charlwood, J.; Birrell, H.; Gribble, A.; Burdes, V.; Tolson, D.; Camilleri, P. (2000)
'A probe for the versatile analysis and characterization of N-linked oligosaccharides'.
Analytical Chemistry **72**: 1453-1461

9 Ludger Technical Note # TN-AP-01: Analysis of 2-AP [2-aminopyridine] labeled glycans

10 Fan, J.Q.; Huynh, L.H.; Lee, Y.C. (1995)
'Purification of 2-aminopyridine derivatives of oligosaccharides and related compounds by cation-exchange chromatography'
Analytical Biochemistry **232**:65-68

11 Iwase, H.; Ishii-Karakasa, I.; Urata, T.; Saito, T.; Saito, T.; Hotta, K. (1990)
'Extraction method for preparing pyridylamino sugar derivates and application to porcine gastric mucus

glycoprotein analysis'.
Analytical Biochemistry **188**: 200-202

12 Tokugawa, K.; Oguri, S.; Takeuchi, M. (1996)
'Large scale preparation of PA-oligosaccharides from glycoproteins using an improved extraction method'.
Glycoconjugate Journal **13**:53-56 (and erratum in Glycoconjugate Journal **13**(4):IV)

Appendix 1 : Troubleshooting Guide

The LudgerTag 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 2-AP Dye / Low Labeling Yield

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 5 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 and the LudgerClean Glycan Cleanup Guide).

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-AP 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 (see protocol step 1 and the LudgerClean Glycan Cleanup Guide).

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 water 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 5 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. Note that the reductive amination reaction is carried out in essentially anhydrous conditions under which loss of sialic acids is minimal.

In general, try to keep samples in solutions in the pH range 5 – 8.5 and avoid exposure to temperatures above 30 ^{o}C. Samples in pH buffered aqueous solutions (with pH between 5 and 8.5) tend to be resistant to acid catalyzed de-sialylation even at temperatures higher than 30^{o}C. However, even then it is wise to err on the

side of caution and keep the samples cool whenever possible.

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 de-sialylation.

L u d g e r ™

LudgerTag™ 2-AP Glycan Labeling Kit

Certificate of Conformity

Kit Specifications

Cat. No. LT-KAP-A2

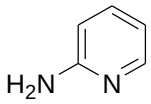
Lot No. A2AS-01

Size The kit contains two sets of labeling reactions, each set typically being sufficient for up 15 separate analytical samples

Application For labeling of glycans with fluorescent 2-AP [2-aminopyridine] dye.

Dye Properties Mass = 94

Fluorescence: λ_{ex} = 295 nm, λ_{em} = 352 nm



Description The kit contains reagents for the conjugation of 2-AP dye to the free reducing end of glycans by a reductive amination reaction.

Number of Samples Typically, up to 15 separate analytical samples per set of labeling reagents.

Amount of Sample Typically, from 1 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 efficiency Typically > 85 % .

Labeling Selectivity Essentially stoichiometric labeling.

Kit Contents

Cat. #	Lot #	Item	Quantity per vial	Number per kit	Appearance
LT-2AP-01	A15O-01	2-AP Dye (2-aminopyridine)	20 mg	2 vials	Solid
LT-DMSO-01	A28K-01	DMSO	350 µl	2 vials	Liquid
LT-ACETIC-01	A27V-01	Acetic acid	200 µl	2 vials	Liquid
LT-CYANOB-02	A13N-02	Sodium cyanoborohydride (Reductant)	12 mg	2 vials	Solid

Declaration of Conformity

This lot conforms to the performance specifications given above.

L u d g e r ™

LudgerTag™ 2-AP Glycan Labeling Kit

Certificate of Conformity

Kit Specifications

Cat. No. LT-KAP-A2

Lot No. A2BO-01

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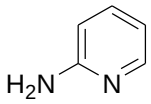

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Cat. #	Lot #	Item	Quantity per vial	Number per kit	Appearance
LT-2AP-01	A15O-01	2-AP Dye (2-aminopyridine)	20 mg	2 vials	Solid
LT-DMSO-01	A28K-01	DMSO	350 µl	2 vials	Liquid
LT-ACETIC-01	A27V-01	Acetic acid	200 µl	2 vials	Liquid
LT-CYANOB-02	A15N-02	Sodium cyanoborohydride (Reductant)	12 mg	2 vials	Solid

Declaration of Conformity

This lot conforms to the performance specifications given above.