LudgerTag™ 2-AB (2-Aminobenzamide) Glycan Labeling Kit

Instruction Guide

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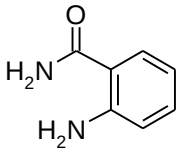
Contents

LudgerTag 2-AB Glycan Labeling Kit Specifications

Cat. No. LT-KAB-Ax (where x denotes pack size)

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

Dye Properties Mass = 136.15
Fluorescence, λ_{ex} = 320 nm, λ_{em} = 420 nm.



Description The kits contain reagents for the conjugation of dye to the free reducing end of the glycan by a reductive amination reaction.

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

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 efficiency Typically > 85 % (dependent on sample).

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-2AB-01	2-AB Dye (2-Aminobenzamide acid)	5 mg
LT-DMSO-01	DMSO	350 µl
LT-ACETIC-01	Acetic acid	200 µl
LT-CYANOB-01	Sodium cyanoborohydride (Reductant)	6 mg

Additional Reagents and Equipment Required

- Heating block, oven, or similar dry heater (a water bath cannot be used) set at 65 ^{o}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 the section on sample cleanup)

Time Line for Labeling

The LudgerTag labeling procedure including the optional post-labeling sample cleanup typically takes between 4 - 5.5 hours:

Procedure	Time	Elapsed Time (hours)
Transfer samples to reaction tube and dry	30 min	0.5
Make up and add labeling reagent	15 min	0.75
Incubate samples	3 hours	3.75
Post-labeling cleanup	1 hour	4.75

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.

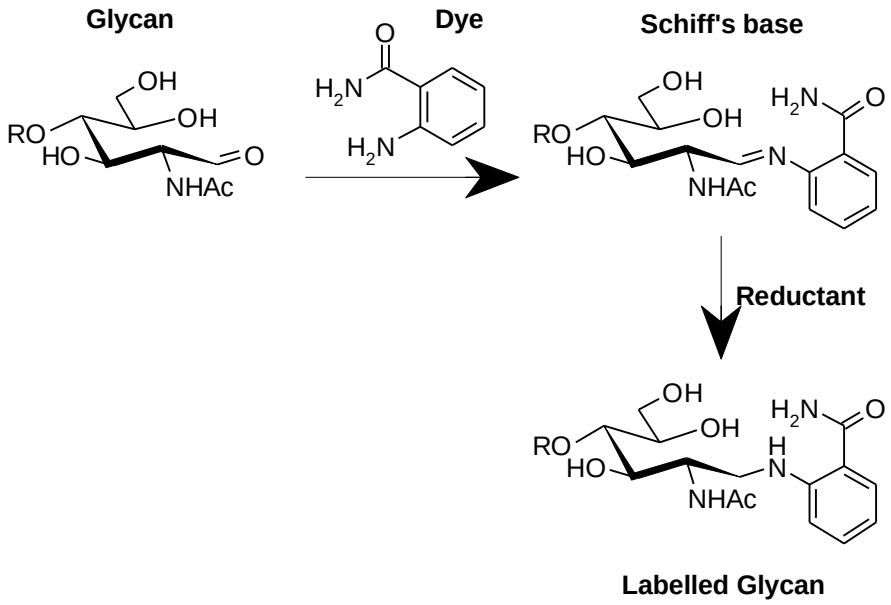


Figure 1: Labeling of a glycan with 2-aminobenzamide acid (2-AB) by reductive amination.

Outline of Labeling Protocol

The LudgerTag™ glycan labeling kits are designed for the fluorophore or chromophore labeling of glycans with a free reducing terminus. Labeled glycans may be followed by either high-sensitivity fluorescence detection or monitoring of UV-absorbance during various chromatographic and structure sequence analyses. These include chromatography on LudgerSep™ HPLC columns and sequencing using exoglycosidases (See refs 1-5, 7).

The outline of the labeling procedure is as follows:

- **Prepare the glycans.**

  Prepare the glycan samples by removing contaminants such as salts and detergents that could interfere with the labeling procedure.

- **Dry the glycans**

  Place the samples in reaction vials and dry down.

- **Prepare labeling reagent**

  Prepare fresh dye labeling solution by mixing reagents in the kit.

- **Add labeling reagent to glycans**

  Add a small amount of labeling solution to each sample.

- **Incubate**

  Incubate the samples to allow the labeling reaction to progress.

- **Post-labeling cleanup**

  After incubation, if required (depending on the subsequent analysis procedures), remove the excess labeling reagents using a straightforward cleanup procedure.

- **Store or Analyse the labeled glycans**

  The labeled glycans are now ready for analysis.

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 LudgerTag 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 LudgerTag labeling is available from Ludger. These are detailed in the LudgerClean Glycan Cleanup Guide [ref 6].

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 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).

Do not subject samples to high temperatures (> 28 ^{O}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).

Preparation of Labeling Reagent

Prepare fresh labeling reagent as follows:

4 Prepare a DMSO-acetic acid mixture

Add 150 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 to between 30^{o}C and 65^{o}C.

5 Add the dye

Add 100 µl of the DMSO-acetic acid mixture to a vial of LudgerTag **2-AB (2-Aminobenzamide Acid) Dye** and mix until the dye is dissolved.

The Cat. # for the dye is LT-2AB-01.

6 Add the reductant

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

The Cat. # for the sodium cyanoborohydride reductant is LT-CYANOB-01.
If the reductant is difficult to dissolve then gently warm the vial for up to four minutes in the 65^{o}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 ml pure water to the vial and mix.
Protect the labeling reagent from exposure to moisture and use within 60 minutes.

Labeling Reaction

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^{o}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^{o}C incubation then the incubation continued.

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.

LudgerClean S 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-Ax, where x denotes the number of cartridges in the kit) 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 LudgerTag 2-AB Labeled Glycans

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

HPLC Analysis

LudgerTag 2-AB labeled glycan mixtures may be separated and analysed by a variety of HPLC (high pressure liquid chromatography) methods including LudgerSep™ HPLC. The LudgerSep columns 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 References 4 and 8.

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.

Enzymatic Analysis

High purity, sequencing grade enzymes (e.g. exoglycosidases) suitable for structural analysis of both N- and O-linked LudgerTag labeled glycans are available from a number of companies.
When selecting glycosidases be especially careful to choose 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 selecting enzymes and reaction conditions for your work.

Mass Spectrometry and Electrophoresis

LudgerTag labeled glycans may also be analysed by mass spectrometry, electrophoresis, and various types of spectroscopy. Please call us for advice on the analysis conditions most suitable for your intended analyses.

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-KAB-Ax-Guide', version v1.0

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'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'.
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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'.
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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 Ludger Technical Note # TN-AB-01: Analysis of 2-AB (2-aminobenzamide acid) labeled glycans

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 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 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 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-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 (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 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. 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.

Ludger™

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Abingdon, Oxford OX14 3EB, UK Tel: +44 (0) 870 085 7011, Fax: +44 (0) 870 163 4620 Email: safety@ludger.com, Website: www.ludger.com
Identification of the substance	Acetic Acid (Cat. # LT-ACETIC-01)
Composition	Solution of acetic acid. Chemical name: Acetic Acid. CAS no. 64-19-7
Hazard identification	Corrosive.
First aid measures	Eyes: irrigate with plenty of water for at least 15 minutes. Skin: wash with soap and water. Ingestion: drink plenty of water. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
Fire fighting measures	Water spray, dry chemical powder or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wash spill site with copious amounts of water.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Colourless liquid.
Stability and reactivity	Avoid contact with bases, oxidising agents and metals.
Toxicological information	Toxic if swallowed, inhaled or absorbed though the skin. High concentrations destructive to upper respiratory tract and eyes.
Ecological information	Data not available.
Disposal considerations	Dilute with excess water, mop up with absorptive material and dispose of according to local regulations.
Transport information	Contact Ludger for transportation information.
Regulatory information	Risk phrases : R35-R21 Safety phrases : S16-S45-S26-S36/37/39

Other information

The advice offered is derived from the currently available information on the hazardous materials in this product or component. Consideration has been made regarding the quantities offered in the pre-dispensed container. The advice offered is, therefore, not all inclusive nor should it be taken as descriptive of the compound generally.

Material Safety Data Sheet

Manufacturer	Ludger Ltd Littlemore Park, Oxford OX4 4SS, UK Tel: +44 (0) 870 085 7011, Fax: +44 (0) 870 163 4620 Email: safety@ludger.com, Website: www.ludger.com
Identification of the substance	DMSO (Cat # LT-DMSO-01)
Composition	Dimethyl sulfoxide. Chemical name: Dimethyl sulfoxide (DMSO). CAS no. 67-68-5
Hazard identification	Irritant.
First aid measures	Eyes: irrigate with plenty of water for at least 15 minutes. Skin: wash with soap and water. Ingestion: drink plenty of water. OBTAIN MEDICAL ATTENTION. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
Fire fighting measures	Water spray or appropriate foam according to surrounding fire conditions. Emits toxic fumes under fire conditions.
Accidental release measures	Wear appropriate protective clothing. As quantities are small absorb with sand or vermiculite and sweep up. Place in bag and hold for disposal. Wash spill site after material pick up is complete.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Colourless liquid.
Stability and reactivity	Avoid contact with acids, oxidising and reducing agents, acid chlorides and phosphorus halides.
Toxicological information	May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information not available.
Ecological information	Data not available.
Disposal considerations	No special requirements. Dispose of according to local requirements.
Transport information	Contact Ludger for transportation information.
Regulatory information	Risk phrases : R36/37/38 Safety phrases : S26-S36-S23

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Identification of the substance	2-AB Dye (Cat # LT-2AB-01)
Composition	2-amino benzamide (Anthranilamide). Chemical name: 2-amino benzamide. CAS no. 88-68-6
Hazard identification	Irritant.
First aid measures	Eyes: irrigate with plenty of water for at least 15 minutes. Skin: wash with soap and water. Ingestion: drink plenty of water. OBTAIN MEDICAL ATTENTION. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
Fire fighting measures	Water spray or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wear appropriate protective clothing. As quantities are small sweep up but avoid raising dust. Place in bag and hold for disposal. Wash spill site after material has been removed.
Handling and storage	Store desiccated at room temperature, avoid exposure to light. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Off-white powder.
Stability and reactivity	Avoid contact with strong oxidising agents.
Toxicological information	May be harmful if swallowed, inhaled or absorbed through skin. May cause skin and eye irritation. Complete toxicological information not available.
Ecological information	Data not available.
Disposal considerations	Dilute with excess water, mop up with absorptive material and dispose of according to local regulations.
Transport information	Contact Ludger for transportation information.
Regulatory information	Risk phrases : R36/37/38 Safety phrases : S26-S36

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Identification of the substance	Sodium Cyanoborohydride (Cat # LT-CYANOB-x)
Composition	Sodium cyanoborohydride. Chemical name: Sodium cyanoborohydride. CAS no. 25895-607
Hazard identification	Flammable, toxic.
First aid measures	Eyes: irrigate with plenty of water for at least 15 minutes. Skin: wash with soap and water. Ingestion: drink plenty of water. Inhalation: move to a well ventilated area and clear nose and throat. If in doubt seek medical advice.
Fire fighting measures	Water spray, dry chemical powder or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wash spill site with copious amounts of water.
Handling and storage	Store at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Off-white powder.
Stability and reactivity	Avoid contact with bases, oxidising agents. Decomposes if exposed to moisture.
Toxicological information	May be *fatal* if swallowed, inhaled or absorbed though the skin. There is less than 10 mg per vial, complete toxicological information not available.
Ecological information	Data not available.
Disposal considerations	Dissolve or mix material with water in a fume cabinet and dispose of according to local regulations.
Transport information	Contact Ludger for transportation information.
Regulatory information	Risk phrases : R23/24/25-R34-R19 Safety phrases : S16-S45-S26-S36/37/39

Other information

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LudgerTag™ 2-AB (2-Aminobenzamide) Glycan Labeling

References

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