Product Guide for LudgerTag™ 2-AA (2-aminobenzoic acid) Monosaccharide Release and Labeling Kit

(Ludger Product Code: LT-MONO-96)

Ludger Document # LT-MONO-96-Guide-v2.4

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Contents

Specifications for LT-MONO-96

Application	For release of neutral and amino monosaccharides from glycoprotein therapeutics and pre-released glycans, and subsequent labeling with 2-aminobenzoic acid (2-AA).
Description	The kit contains reagents for the release of monosaccharides from glycoprotein biopharmaceuticals and standards. Released monosaccharides have a free reducing terminus to allow fluorescent tagging by reductive amination. **There are two hydrolysis acids provided. 2 molar trifluoroacetic acid (2M TFA) and 6 molar hydrochloric acid (6M HCl). Typically, for a pilot study, we recommend using both these acids on separate replicates of your samples as we have found that 2M TFA is good for releasing neutral monosaccharides but is less effective with the N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) monosaccharide release for which we recommend using 6M HCl. After a pilot study it may be determined that the use of just a 6M HCl hydrolysis step may be sufficient for quantitative analysis of your glycoprotein. Hydrochloric acid as well as providing more effective release of GlcNAc may also aid core fucose release and core mannose release; however these latter monosaccharides are subject to degradation by HCl so care should be taken to perform hydrolysis in a consistent manner and we recommend a simultaneous hydrolysis of the monosaccharide standards for calibration purposes.**
Number of Samples	The kit contains reagents and materials for up to 96 glycoprotein samples analysed in parallel or two sets of 48 samples.
Amount of Sample	Typically, around 50 µg of glycoprotein per sample dependent on levels of glycans present which should be determined in a pilot study.
Suitable Samples	Biopharmaceutical glycoproteins.
Storage	Store the whole kit at 4°C in the dark. Once monosaccharide standards are dissolved in solvent we recommend storing them at -20°C. Protect from sources of heat, light, and moisture. Use kit within 6 months of purchase.
Shipping	The product should be shipped at ambient temperature, but can be stored at 4°C for up to 6 months.
	For research use only. Not for human or drug use.

Kit Contents

The kit contains the following materials and reagents:

Cat. #	Item	Quantity
LT-2MTFA-01	Trifluoroacetic acid	2
LT-6MHCL-01	Hydrochloric acid	2
LT-NAOAC-01	Sodium acetate	2
LT-NBM-01	Labeling solvent	2
LT-2AA-02	2-aminobenzoic acid	2
LT-CYANOB-03	Sodium cyanoborohydride	2
CM-MONO-MIX-10	10 nmols each of glucosamine, galactosamine, galactose, glucose (dextrose) mannose and fucose	4
CM-XYL-100	100 nmol of xylose	2

Additional Reagents and Equipment Required

- Pure water: resistivity above 18 MΩ-cm, particle free (>0.22 μm), TOC <10 ppb
- Dialysis membranes, PD10 columns or similar for removal of salts and detergents from your glycoprotein samples*
- Vials - Screw cap polypropylene vials for hydrolysis. 0.5 mL volume size is ideal. Vials lids should seal tightly and have O-rings to prevent escape of acid vapour. We do not recommend the use of snap cap type vials such as used for PCR unless they are designed to fit securely without clamping.
- Oven for incubation stages (recommended) or heating block. Note that the use of a heating block often results in some sample solvent evaporation into the vial lid which may reduce the effectiveness of sample hydrolysis or labeling.
- Centrifugal evaporator (e.g. Genevac, Savant or similar)
- Vortexer and sonicator.
- Pipettes
- LudgerSep™ R2 HPLC column (Cat No.LS-R2-4.6x150) or LudgerSep uR2 UHPLC column (Cat. No. LS-UR2-2.1x50).
- For the HPLC solvent: Butylamine, >99.5%
  Tetrahydrofuran, anhydrous >99.9%, BHT inhibitor
  Orthophosphoric acid about 85% concentration
  Acetonitrile
  Or, LudgerSep™ R BPT solvent ten times concentrate (Cat. No. LS-R-BPTX10).

- HPLC sample filters

* Optional - depending on your sample. High levels of salts may inhibit fluorophore labeling. The xylose internal control can be used as a guide as to whether high salt labeling inhibition is occurring.

Safety and Handling

- Please read the Material Safety Data Sheets (MSDS) for all chemicals used (see Appendix 2).

- All processes involving the kit reagents should be performed using appropriate personal safety protection – safety eyeglasses, good quality chemically resistant gloves (e.g. nitrile), etc. - and where appropriate in a laboratory fume cupboard.

- Ensure that any glass, plastic ware 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.

- Once individual vials of reagents are opened, their contents should be used immediately and excess then discarded according to local safety rules.

Time Line for Procedure

The LudgerTag™ monosaccharide release and labeling procedure typically takes 14 hours (excluding HPLC):

Procedure	Approx Time
Dispense and dry samples	4h
(samples can be stored at -20 °C until required)	
Hydrolyse samples	3h
Cool and dry samples	4h
(samples can be stored at -20 °C until required)	
Add labeling reagents	1h
Chemically label	1h
Dilute samples	1h

Outline of Protocol

1. Dispense and dry samples

2. Hydrolyse samples

3. Cool and then dry samples

4. Add labeling reagents

5. Chemically label samples

6. Dilute samples

7. HPLC analysis of samples

Instructions for Use

Dispense and dry samples

1. Dispense 50 µg of each glycoprotein sample into a 0.5 mL screw cap polypropylene vial in triplicate.

Two samples are required for each analysis – one for 2M TFA hydrolysis and one for 6M HCl hydrolysis.

Ideally each solution should be no greater than 200 µL in volume to prevent sample loss out of the vial during the centrifugal drying process. The use of 0.5 mL screw cap vials with O-rings in the lids is recommended (snap cap lid type vials may fail during the hydrolysis step).

2 Sample Blank - Dispense equivalent volume of sample buffer into a 0.5 mL screw cap polypropylene vial in triplicate.

3 Dry samples by centrifugal evaporation

Do not apply heat. Samples should be dry within three hours dependent on vacuum efficiency.

Hydrolyse samples

4. To dried sample add 200 µL LT-2MTFA-01 – For neutral monosaccharide analysis (Gal, Man, Glc, Fuc, Xyl)

Vortex for 10 seconds and tighten cap to ensure no evaporation from tube during heating step.

At this stage you should also add acid to the dry 10 nmol monosaccharide standards (CM-Mono-Mix-10).

5. To dried sample add 200 µL LT-6MHCl-01 – For N-acetylgalactosamine and N-acetylglucosamine analysis (GalNAc, GlcNAc)

6M HCl is more effective at releasing GalNAc and GlcNAc (and possibly core fucose and mannose) monosaccharides than 2M TFA but will partially destroy the neutral monosaccharides.

6. Place vials in oven

Heat samples at 100° C for 3 hours in an oven. The samples should be re-mixed after 30 min to ensure dissolution of the glycoprotein in the acid. If a hot plate is used instead of an oven smaller vials will need to be used or a larger volume of acid to prevent all the acid from evaporating and condensing on the sample vial lid and not sufficiently hydrolysing the sample.

Please note that there are many different hydrolysis conditions reported in the scientific literature and we recommend that you optimise these conditions with your own glycoproteins.

Cool and then dry samples

7. Remove vials from oven and allow them to cool.

Centrifuge to ensure all sample is in the vial and not the cap.

8. Add xylose internal standard to each sample.

We recommend the use of the xylose monosaccharide as an internal standard. We provide a 100 nmol amount of xylose for this purpose. Dissolve the xylose in a set amount of water (e.g. 200 µl), mix thoroughly to ensure complete dissolution and then add a set amount to each test sample (e.g. 10 nmol or 20 µl dissolved sample). Often the use of xylose is more effective in a full study after a preliminary investigation has already been performed on the samples and any presence of xylose and the absolute levels of monosaccharides in the test samples have been determined. Xylose is not recommended as a standard for proteins expressed by plant-cell based expression systems.

9. Loosen vial caps and evaporate the acid in a centrifugal evaporator

This stage should take up to 4 hours. After 2 hours of drying check that vial cap O-rings are not sealing the tube preventing sample drying. Many samples will dry to a dark brown or black spot.

Add labeling reagents

10. Add 50 µL LT-NaOAc-01 solution to each sample

Ensure samples dissolve thoroughly – quantitation of the monosaccharide levels within the sample is dependent on good dissolution of the sample at this stage. We recommend briefly vortexing samples followed

by sonication for 15 minutes, to solubilise samples. Centrifuge samples briefly to remove any solution from the vial cap. **One of the greatest sources of error within monosaccharide analysis is insufficient dissolution of sample.**

11. Make up the 2-AA solution

Add 2750 µL of the LT-NBM-01 solution to the LT-2AA-02 vial. Ensure the dye dissolves to completion.

12. Make up the LT-CYANOB-03 solution

Add 2750 µL of LT-NBM-01 solution to the sodium cyanoborohydride vial and ensure it dissolves to completion.

13. Add 2-AA and sodium cyanoborohydride mixes to each sample

Add 50 µL of the 2-AA dye followed by 50 µl of sodium cyanoborohydride to each of the samples and controls. Cap and vortex sample for about 10 seconds. Pre-mixing the 2-AA dye and sodium cyanoborohydride before adding to the sample can result in a large amount of precipitant being formed.

Chemically label samples

14. Preheat oven/hot plate to 80 °C

Note that we recommend the use of an oven as this prevents any solution condensing in the sample cap.

15. Incubate samples for 45 minutes at 80° C

Ensure that the vial lids are tight.

Dilute samples

16. Remove samples from incubator and allow to cool

Cool the samples to room temperature and then briefly spin in a centrifuge to remove condensate from vial cap.

17. Dilute samples

Once cooled and centrifuged the 2-AA labeled samples are ready for analysis by HPLC on the LudgerSep™R2 column or UHPLC analysis on the LudgerSep™uR2 column. At this stage the labeling solutions are too concentrated to be injected onto the HPLC column. Samples are diluted into the butylamine/ortho-phosphoric acid/tetrahydrofuran (BPT) HPLC running solvent. For the monosaccharide standards we recommend diluting the samples 100 fold, but exact dilutions are dependent on the sensitivity of the fluorescence detector and the glycan content of the glycoprotein. See the calibration curve section of this document. As a guide, antibody samples should be diluted 20 to 50 fold as there is often only two glycosylation sites per molecule. Bovine fetuin glycoprotein standard (Cat. No. GCP-Fet-50) is usually run at 100 fold dilution, where fetuin is approximately 20% w/w glycan. After samples dilution we recommend sonicating the samples for 30 minutes to remove any gas produced at the dilution stage. Vials can remain capped during sonication. If a sonicator is not available samples can be left for approximately 12 hours after dilution and then lightly mix to remove bubbles. No further gas release will occur after 12 hours.

HPLC analysis of samples

For analysis of the 2-AA labeled monosaccharides, the LudgerSep™ R2 column (Cat No. LS-R2-4.6x150) gives very good separation of the seven main monosaccharides found in most N-link and O-link glycans. This column is optimal for HPLC based separations, **however an 8 minute separation can now be performed using ultra high pressure liquid chromatography (UHPLC) equipment. If you have an UHPLC system we recommend using the LudgerSep™ uR2 column (Cat No. LS-UR2-2.1x50) and LudgerSep™ R BPT solvent concentrate (Cat. No. LS-R-BPTX10). Please refer to the LudgerSep-uR2 guide for detailed installation instructions (available on our website).**

Solvents

The glycan analysis gradients in this guide are based on the following solvents:

Solvent A : purified water based solvent of 0.2 % butylamine (2 mL per litre), 0.5 % phosphoric acid (5 mL per litre), 1 % tetrahydrofuran (10 mL per litre) (henceforth called BPT solvent).

Solvent B : 50 % acetonitrile : 50 % solvent A

We have also tested acetonitrile free solvent systems. This requires a slightly different gradient. Please enquire for further details. Column should be stored in 30% solvent B long term.

Gradient

Column temperature: 30 °C
Fluorescence detector settings : Excitation wavelength: 360 nm, Emission wavelength: 425 nm

Time (min)	**%B**	**Flow Rate** (ml/min)
0.00	7	0.8
7.00	7	0.8
25	17	0.8
26	100	0.8
36	100	0.8
37	7	0.8
45	7	0.8

An 8 minute gradient can be used on UHPLC systems in conjunction with the LudgerSep™ uR2 column (Cat No. LS-uR2-2.1x50).

Calibration Curve

We recommend a five point calibration curve to be used for the monosaccharide standards. If you have used both HCl and TFA hydrolysis conditions you will need to perform a calibration curve for a set of monosaccharide standards hydrolyzed with each acid.
Dilute the hydrolyzed monosaccharide mixtures into BPT solvent. We recommend the following dilutions, but these may vary dependent on the glycosylation levels in your sample. Always run a standard curve that allows you to place your sample results within the curve. Pipetting larger volumes than 5 µl is generally more accurate.

1in 100 (25 µl hydrolyzed monomix standard + 2475 µl BPT)
1 in 125 (20 µl hydrolyzed monomix standard + 2480 µl BPT)
1in 167 (15 µl hydrolyzed monomix standard + 2485 µl BPT)
1in 250 (20 µl hydrolyzed monomix standard + 4980 µl BPT)
1 in 500 (10 µl hydrolyzed monomix standard + 4990 µl BPT)

Sonicate samples to remove gas bubbles. The vial lid does not need to be loosened for this stage.

Data Interpretation

HPLC analysis of 2-AA monosaccharides is a robust method for determining monosaccharide levels in your glycoprotein sample. There are some important issues that need to be understood to enable effective data interpretation.

1. Each monosaccharide has a different fluorescence yield per nmol. A standard curve of monosaccharide concentration versus fluorescence needs to be determined.
2. Free dye is not removed from the samples after 2-AA labeling to ensure no monosaccharides are lost in a dye clean-up stage. Free dye elutes as a large off-scale peak at about 15 minutes (Figure 1), **and also several large peaks after 28 minutes**.
3. N-acetylglucosamine and N-acetylgalactosamine are both de-N-acetylated during the acid hydrolysis step of this process to produce glucosamine and galactosamine respectively.
4. Glucosamine is subject to an approximate 4 % epimerisation resulting in mannosamine (Figure 2). Mannosamine elutes immediately before the galactosamine peak, with no baseline resolution.
5. Galactosamine also produces an epimer peak (Figure 1 – small peak immediately after GalN), typically smaller than 2 %. However this epimer peak often elutes with the free dye peak.
6. The use of xylose as an internal standard (Figure 3) allows samples to be compared directly. This allows compensation for any pipetting/sample preparation errors that may have occurred during sample processing.
7. Sample stability – we recommend keeping labeled samples in the freezer at -20 °C if you intend to store them for longer than one week.

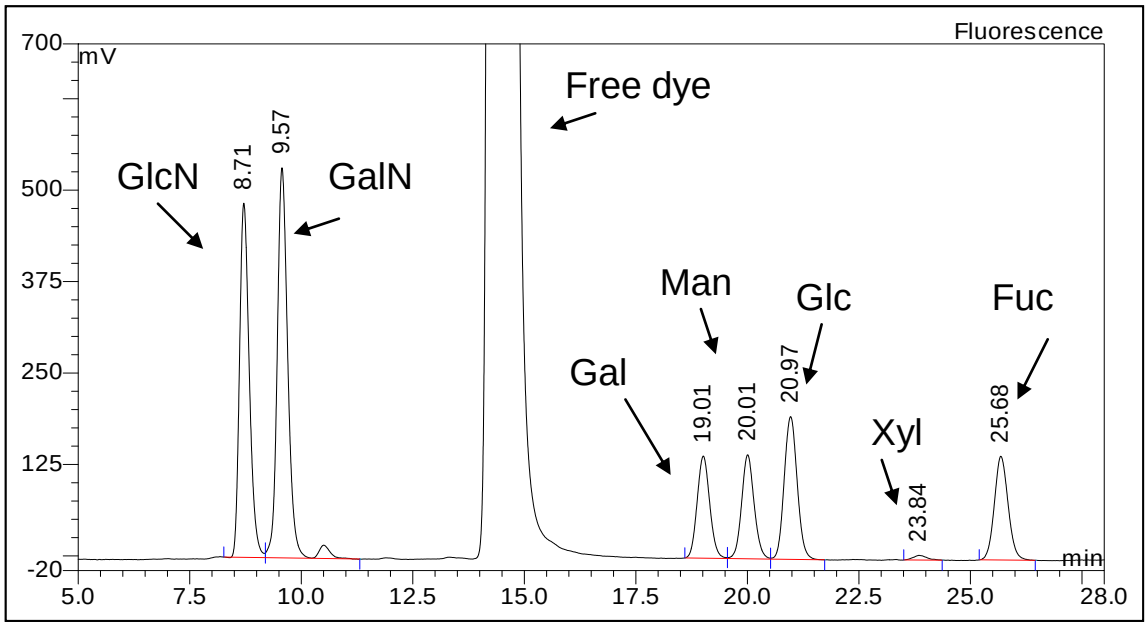

Figure 1: 2-AA fluorescence chromatogram of monosaccharides glucosamine (GlcN), galactosamine (GalN), galactose (Gal), mannose (Man) and fucose (Fuc). Glucose elutes immediately after the Man peak. Xylose elutes before the Fuc peak.

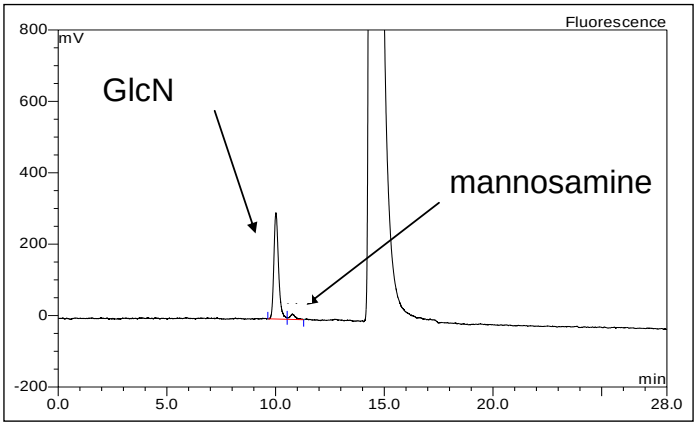

Figure 2: 2-AA fluorescence chromatogram of monosaccharide glucosamine (GlcN). This chromatogram shows the epimer peak from GlcN.

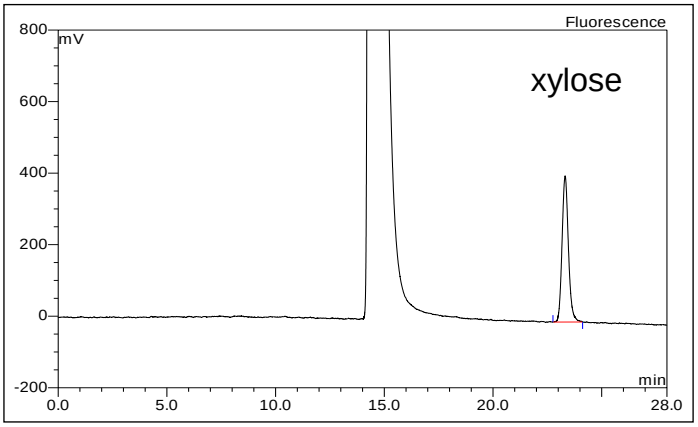

Figure 3: 2-AA fluorescence chromatogram of monosaccharide internal reference xylose (Xyl).

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-MONO-96-Guide-v2.4

Appendix 1 : Troubleshooting Guide

The following is a guide to the most likely problems associated with monosaccharide analysis, possible causes, and solutions.

Low Yield or High Sample to Sample Variability

The sample was incompletely solubilized.

The hydrolysed samples can be difficult to dissolve in 1 % sodium acetate prior to labeling. In the case of a black/brown hydrolysed sample it is clear when the sample has not dissolved, however, not all sample pellets are readily visible. To ensure good solubility of the sample, shake/sonicate the sample vials rather than just vortexing them. Once dissolved, centrifuge the sample briefly.

Variability in Pipetting

Ensure that pipettes are calibrated and that the pipette tips used are maximum recovery type.

Diluted samples not sufficiently degassed.

Gas production/displacement occurs in the samples shortly after dilution. We recommend running the samples on the HPLC after this process of degassing has finished (up to 12 hours after dilution if left to stand in a refrigerator, or after 30 minutes in a sonication bath) otherwise air bubbles can be injected onto the HPLC column causing large sample to sample variation. The gas released is low enough that sample vial caps do not need to be loosened.

Precipitation in sample vials

Large amount of dye/reductant excess

In order to get good labelling efficiency a large molar excess of dye and reductant to free monosaccharide can be used. This can sometimes result in a cloudy precipitate forming in samples. Reducing the concentration of dye and reductant in the labelling reaction may help to prevent the precipitation occurring.

Large variation in retention times for peaks on HPLC

Old solvents

Ensure that HPLC solvents are made up fresh for each chromatography run.

Inconsistent solvent preparation

Ensure that solvent components are dispensed accurately. Sometimes pipette tips drip because they are not compatible with the solvent being dispensed. Weighing of solvent components is often used as an alternative to the use of volumes when dispensing.

Appendix 2: Material Safety Data Sheets

Material Safety Data Sheet: LT-2MTFA-01

Manufacturer	Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com
Identification of the substance	2M Trifluoroacetic acid (aq) (Cat # LT-2MTFA-01)
Composition	Trifluoroacetic acid and water Chemical name: Trifluoroacetic acid. CAS no. 76-05-01
Hazard identification	Both liquid and vapor can cause burns to all parts of the body.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures

Water spray or appropriate foam according to surrounding 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 or 4°C. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal Protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.
Physical and chemical properties	Colourless liquid.
Stability and reactivity	Stable under normal temperatures and pressures.
Toxicological information	May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information 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 :

R 35 Causes severe burns.

R 20 Harmful by inhalation.

R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases :

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 27 Take off immediately all contaminated clothing.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S 9 Keep container in a well-ventilated place.

S 28A After contact with skin, wash immediately with plenty of water

Material Safety Data Sheet: LT-6MHCl-01

Manufacturer	Ludger Ltd, Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620, www.ludger.com
Identification of the substance	6M Hydrochloric acid (aq) (Cat # LT-6MHCl-01)
Composition	Hydrochloric acid and water Chemical name: Hydrochloric acid. CAS no. 7647-01-0
Hazard identification	Both liquid and vapor can cause burns to all parts of the body.

First aid measures

Eyes: irrigate with plenty of water for at least 15 minutes.

Skin: wash with plenty of water for at least 15 minutes

Ingestion: drink a cupful of water.

Inhalation: move to a well ventilated area and clear nose and throat.

Get medical aid immediately.

Fire fighting measures

Water spray or appropriate foam according to surrounding 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 or 4°C. Handle in accordance with Good Laboratory Practice.
Exposure Controls / Personal Protection	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with GLP.
Physical and chemical properties	Colourless liquid.
Stability and reactivity	Stable under normal temperatures and pressures.
Toxicological information	May be harmful if swallowed, inhaled or absorbed through skin. May cause irritation, complete toxicological information 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 :

R 23 Toxic by inhalation.

R 24 Toxic in contact with skin.

R 25 Toxic if swallowed.

R 34 Causes burns.

R 36 Irritating to eyes.

R 37 Irritating to respiratory system.

R 38 Irritating to skin.

Safety phrases :

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36 Wear suitable protective clothing.

S 37 Wear suitable gloves.

S 39 Wear eye / face protection.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Material Safety Data Sheet: LT-CYANOB-03

Identification of the substance	Sodium Cyanoborohydride (Cat # LT-CYANOB-03)
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 or 4°C. 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

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: CM-Mono-Mix-10/CM-Xyl-100

Identification of the substance	Ludger monosaccharides
Composition	Each vial of CM-Mono-Mix-10 contains 10 nmols each of monosaccharide: CAS nos. Glucosamine Hydrochloride 66-84-2 Galactosamine Hydrochloride 1772-03-8 Galactose 3646-73-9 Mannose 3458-28-4 Fucose 2438-80-4 Glucose (Dextrose) 921-60-8 CM-Xyl-100 contains: CAS no. Xylose 58-86-6
Hazard identification	Possible allergic reaction to material if inhaled, ingested or in contact with skin.
First aid measures	In case of contact: Eyes: irrigate with plenty of water. 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	Non hazardous. Water spray or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wash spill site with copious amounts of water.
Handling and storage	Store desiccated at -20°C, or 4°C for up to 6 months. 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 dried material, freely soluble in water.
Stability and reactivity	Not combustible.
Toxilogical information	Toxicological, carcinogenic and mutagenic properties have not been investigated.
Ecological information	Data not available.
Disposal considerations	No special requirements. Dispose of according to local requirements.

Transport information	Contact Ludger Ltd for transportation information.
Regulatory information	Data not available.
Contact Details of Manufacturer	Ludger Ltd Culham Science Centre, Abingdon, Oxfordshire OX14 3EB, UK Tel: +44 870 085 7011 Email: info@ludger.com Web: www.ludger.com
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: LT-2AA-02

Identification of the substance	2-AA Dye (Cat # LT-2AA-02)
Composition	2-amino benzoic (Anthranilic acid). Chemical name: 2-amino benzoic acid. CAS no. 118-92-3
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 or 4°C, 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/S39

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: LT-NaOAc-01

Identification of the substance	Sodium acetate solution (Cat # LT-NaOAc-01)
Composition	sodium acetate in purified water. Chemical name: sodium acetate. CAS no. 127-09-3
Hazard identification	May cause irritation to skin, eyes and respiratory tract. Not regulated as a hazardous material.
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 solution at 4^{o}C, 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	Colourless solution.
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	Safety phrases: S 24/25

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: LT-NBM-01

Identification of the substance	Labeling solvent
Composition	Salts in organic solvent. Risk phrases: R60, R11 R23 R24 R25 R39.
Hazard identification	May cause irritation to skin, eyes and respiratory tract. Highly flammable. Harmful by inhalation, absorption through the skin and to the environment – dispose of using specialist chemical services. 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. 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, carbon dioxide or appropriate foam according to surrounding fire conditions.
Accidental release measures	Wear appropriate protective clothing. Quantities supplied here are small. For large volumes, shut off all sources of ignition. Use nonsparking tools. Clean-up using chemical spillage kits containing materials, such as sand.
Handling and storage	Store solution at 4^{o}C, avoid exposure to light. Keep away from heat and open flames. 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 solution.
Stability and reactivity	Stable. Flammable. Avoid oxidizing agents. May react violently with acids, potassium, acid chlorides, acid anhydrides, oxidizing agents, reducing agents and alkali metals. Incompatible with water, strong bases, alkali metals. Moisture sensitive. Hygroscopic.
Toxicological information	May be harmful/toxic if swallowed, inhaled or absorbed through skin. May cause skin and eye irritation. To the best of our knowledge complete toxicological information of this solution mix is 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	UN No 1230. Packing group II. Hazard class 3 (6.1)
Regulatory information	Safety phrases: S 16, 22, 24, 25, 26, 36, 37, 38, 45

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.