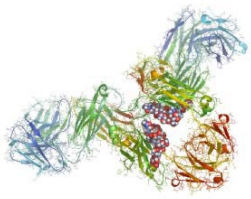

Ludger

LudgerTag™ DMB

(1,2-diamino-4,5-methylenedioxybenzene.2HCl)

Sialic Acid Labeling Kit

Instruction Guide

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Contents

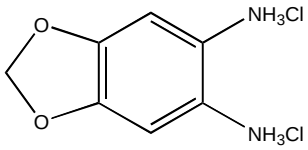
LudgerTag DMB Glycan Labeling Kit Specifications

Cat. No. LT-KDMB-A1

Application: Labeling of sialic acids with 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) for fluorophore detection of chromatographically separated sialic acid variants.

Dye Properties: Relative Molecular Mass = 120.11 $gmol^{-1}$
Fluorescence, $?_{ex}$ = 373 nm, $?_{em}$ = 448 nm.

Structure:



Synonyms: DMB
1,2-Diamino-4,5-methylenedioxybenzene Dihydrochloride
1,3-Benzodioxole-5,6-diamine Dihydrochloride
5,6-Diamino-1,3-benzodioxole Dihydrochloride

Description: The kit contains reagents for the release of sialic acids from glycoproteins and the subsequent conjugation of a dye by a reductive amination reaction.

Number of Samples: Typically, up to 22 separate analytical samples per set of labeling reagents (including a sialic acid reference panel, N-acetyl neuraminic acid and N-glycolyl neuraminic acid)

Amount of Sample: From 10 pmol up to 2.5 nmol sialic acids per sample.

Suitable Samples: Any sialic acid released from a glycoprotein, glycopeptide or glycan can be labeled.

Labeling efficiency: Typically >85 % (dependent on sample).

Labeling Selectivity: Essentially stoichiometric labeling.

Storage: Store at -18^{O}C 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 except acetic acid 2 molar, which you get two vials of:

Cat. No.	Item	Quantity
LT-DMB-01	DMB Dye	~ 0.7 mg
LT-ACETIC2M-01	Acetic Acid 2 Molar	1.1ml
LT-MERCAPTO-01	Mercaptoethanol in Acetic acid (1.4 Molar)	500 µl
LT-NADITHIO-01	Sodium Dithionite (Reductant)	~ 4 mg
LT-SIALICREF-01	Sialic Acid Reference Panel	~ 0.125 nmol
CM-NEUAC-01	N-acetylneuraminic acid quantatative std	1 nmol
CM-NEUGC-01	N-glycolylneuraminic acid qualitative std	1 nmol

Additional Equipment Required

Heating block, oven, or similar dry heater (a water bath cannot be used) set at 80^{O}C for the sialic acid release step and at 50^{O}C for the sialic acid labelling reaction
Reaction vials (e.g. polypropylene microcentrifuge vials)

Additional Reagents Required

Analytical grade water eg. MilliQ

Time Line for Labeling

The Ludger labeling procedure, including the release of sialic acids from the samples to be analysed, typically takes ca. 7 hours:

Procedure	**Typical Time**
Transfer samples to reaction tube and dry	60 min
Addition of sialic acid release agent	15 min
Incubate samples	2 hours
Cool and transfer samples to reaction tube	15 min
Make up and add labeling reagent	30 min
Incubate samples	3 hours

Reaction Mechanism: Formation of Substituted Quinoxaline: DMB Labeled Sialic Acid

The labeling reaction involves a two-step concerted process, which is carried out *in situ* (see Figure 1a and 1b):

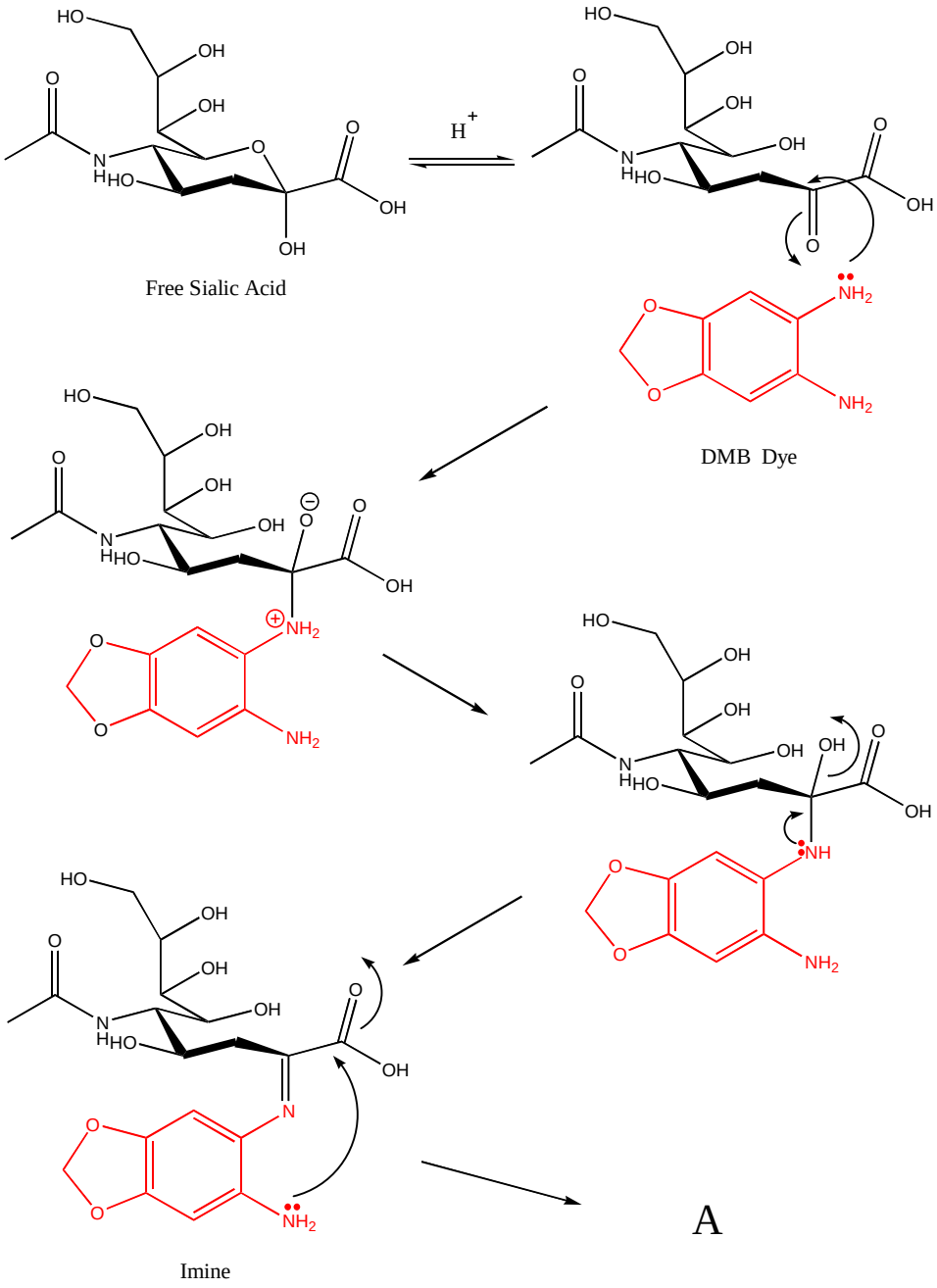


Figure 1a: Labeling of a sialic acid with DMB

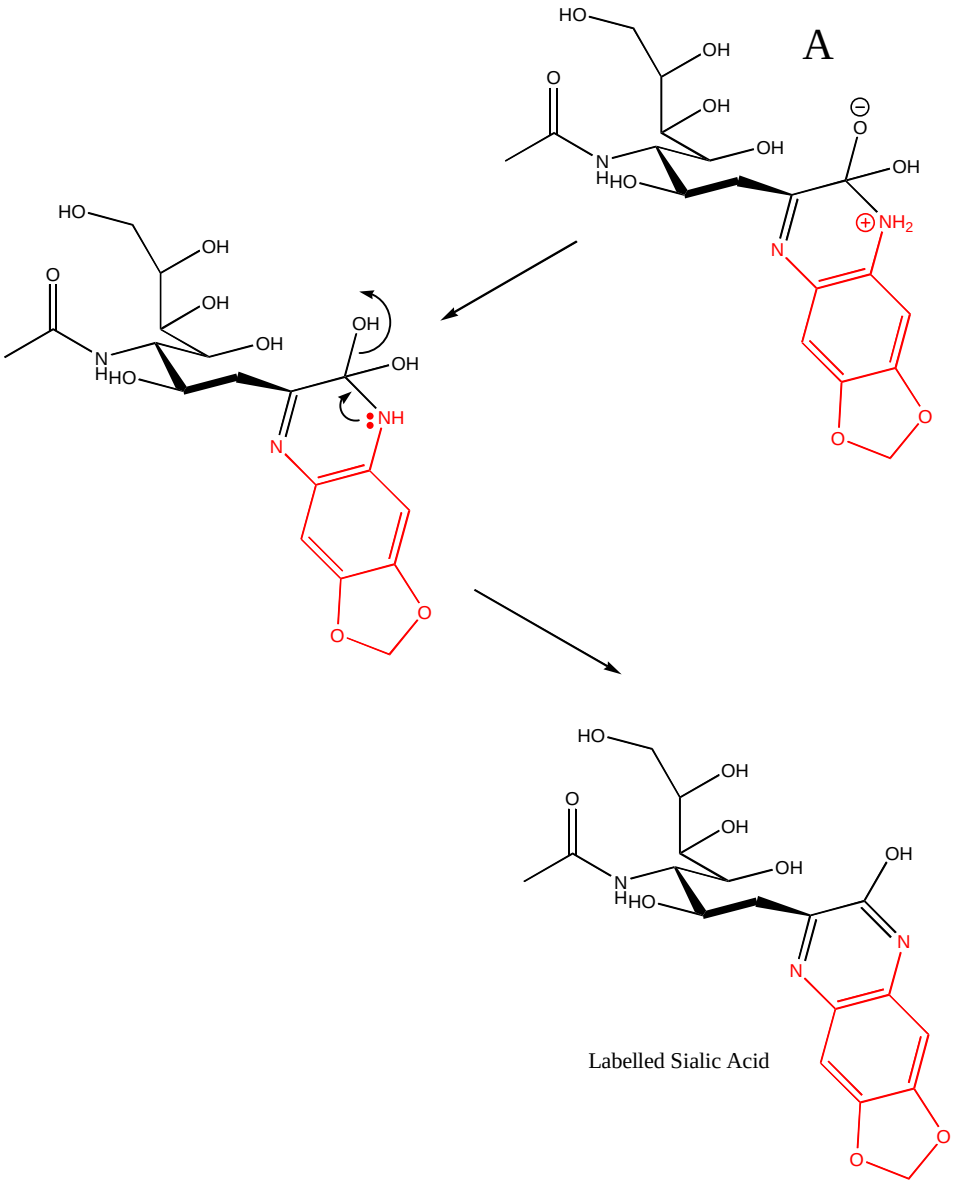


Figure 1b: Labeling of a sialic acid with DMB

Labeling Kit Introduction

The LudgerTag DMB labeling kit is designed for the fluorophore or chromophore labeling of sialic acids. Labeled sialic acids 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 the LudgerSep™ R1 HPLC column (Cat. No. LS-R1-4.6x150).

Outline of Ludger DMB glycan labeling protocol

Stage 1:

Dry down glycoprotein/glycopeptide/glycans to be analaysed for sialic acid content

Add Sialic acid release reagent (where appropriate)

Incubate

Stage 2:

Prepare labeling reagent

Add labeling reagent to sample

Incubate

Terminate Reaction

Store or analyse the labeled sialic acids

Sialic Acid Release

1 Glycoprotein/glycopeptide/glycans Preparation

Glycoproteins/glycopeptides/glycans must be dried before they can be labelled. It is preferable for the dried glycoproteins/glycopeptides/glycans to be in a small vial, eg 0.5ml polypropylene microcentrifuge vial, but the procedure can also work efficiently in 5ml glass screw capped vials.

2 Sialic Acid Release

Add 100 µl of the sialic acid release reagent, LT-ACETIC2M-01, to the sialic acid containing sample and incubated at 80^{O}C for 2 hours.

3 Sialic Acid Transfer

The sample is cooled and vortexed. 5 µl is removed and transferred to a separate 0.5ml polypropylene microcentrifuge vial to be labeled with DMB.

Preparation of Labeling Reagent

4 Prepare Labeling Reduction Solution

Add 440µl of the mercaptoethanol solution, LT-MERCAPTO-01, to the vial of sodium dithionnite (reductant), LT-NADITHIO-01, and mix until the reductant is completely dissolved.

5 Prepare final Labeling Solution

Add the entire contents of the reduction solution, to the vial of Ludger DMB Dye, LT-DMB-01, and mix until the dye is dissolved.

NB: Protect the labeling reagent from exposure to moisture and light and use within 60 minutes

Labeling Reaction

7 Add labeling reagent to samples

Add 20 µl of labeling reagent to each sample, including Sialic Acid Reference Panel, LT-SIALICREF-01, N-acetylneuraminic acid, CM-NEUAC-01, and N-glycolylneuraminic aci, CM-NEUGC-01, cap the microtubes, 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 50^{O}C and incubate for 3 hours in the dark.

9 Terminate Reaction

Terminate the reaction by adding 500 µl of water.

Store at 4^{O}C in the dark until subsequent analysis.

Analyses should be carried out within a few hours of termination.

HPLC Analysis

We recommend that DMB labeled sialic acids are analyzed using high performance liquid chromatography (HPLC). The LudgerSep R1 HPLC column (Cat. No. LS-R1-4.6x150mm) is ideally suited for this purpose. The DMB labeled sialic acid reference panel is an excellent sample to run on this column to ensure DMB labelling has performed within your in-house specifications. An example DMB sialic acid reference panel chromatogram is shown below.

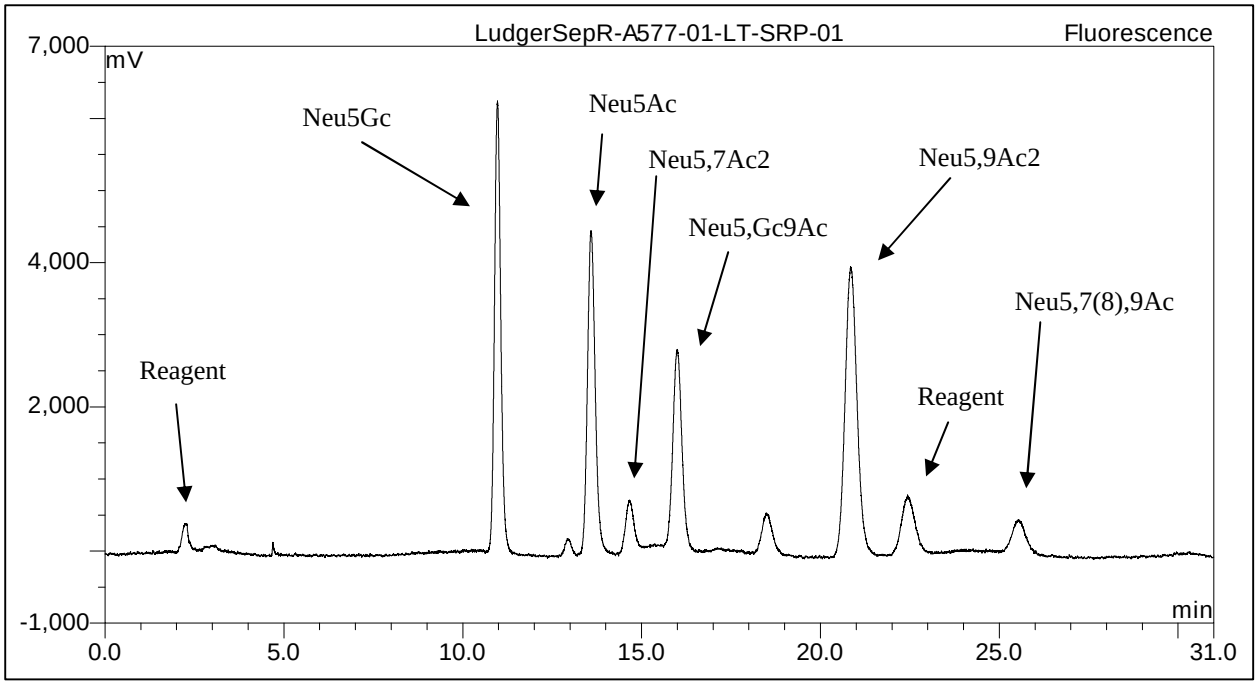


Figure 2: DMB Labeled Sialic Acid Reference Panel Run on the LudgerSep R1 HPLC column (Cat. No. LS-R1-4.6x150mm)

Separation Conditions:

An isocratic gradient at room temperature over a 30 min period is recommended.

Flow rate:	0.5 ml/min
Solvent:	Methanol:Acetonitrile:Water (7:9:84)

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-KDMB-A1-Guide', revision v2.2

Appendix A1: Preparation of NeuAc Standard Curve

The preparation of the NeuAc standard curve involves labelling the vial of NeuAc with DMB and then performing a set of serial dilutions.

1 After the DMB labelling reaction, the DMB labelled NeuAc (DMB-NeuAc) is in a volume of 500µl. Pipette 250 µl of the DMB-NeuAc solution into each of two new vials. Label one vial, vial A, 50 % DMB-NeuAc (0.5 nmol), and the second vial, vial B, which will be taken through the serial dilutions.

2 Add 250 µl of water to vial B and vortex the vial to ensure thorough mixing.

3 Remove 250 µl of the DMB-NeuAc solution from vial B and pipette into a vial labelled vial C. Both vials now contain 0.25 nmol DMB-NeuAc. Vial B is now the 25 % DMB-NeuAc concentration (0.25 nmol) and vial C is to be taken through to the next step.

4 Add 250 µl of water to vial C and vortex the vial to ensure thorough mixing.

5 Remove 250 µl of the DMB-NeuAc solution from vial C and pipette into a vial labelled vial D. Both Vials now contain 0.125 nmol DMB-NeuAc. Vial C is now the 12.5 % DMB-NeuAc concentration (0.125 nmol) and vial D is to be taken through to the next step.

6 Add 250 µl of water to vial D and vortex the vial to ensure thorough mixing.

7 Remove 250 µl of the DMB-NeuAc solution from vial D and pipette into a vial labelled vial E. Both Vials now contain 0.0625 nmol DMB-NeuAc. Vial D is now the 6.25 % DMB-NeuAc concentration (0.0625 nmol) and vial E is to be taken through to the next step.

8 Add 250 µl of water to vial E and vortex the vial to ensure thorough mixing.

9 Remove 250 µl of the DMB-NeuAc solution from vial E and pipette into a vial labelled vial F. Both Vials now contain 0.03125 nmol DMB-NeuAc. Vial E is now the 3.125 % NeuAc concentration (0.03125 nmol) and vial F is to be taken through to the next step.

10 Add 250 µl of water to vial F and vortex the vial to ensure thorough mixing.

11 Remove 250 µl of the DMB-NeuAc solution from vial F and pipette into a vial labelled vial G. Both Vials now contain 0.015625 nmol DMB-NeuAc. Both vials are now 1.563 % DMB-NeuAc concentration.

12 Vials (A, B, C, D, E, F and G) are ready for the HPLC analysis to get the results to plot a standard curve.

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Abingdon 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:	DMB Dye (Cat # LT-DMB-01)
Composition:	1,2-diamino-4,5-methylenedioxybenzene dihydrochloride CAS no. 81864-15-5
Physical properties:	MP/MP Range 247 °C Colour: Slightly beige/white semi crystalline solid
Hazard identification:	Irritating to eyes, respiratory system and skin.
First aid measures:	EYE CONTACT: In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. SKIN CONTACT: In case of skin contact, flush with copious amounts of water for at least 15 minutes INGESTION: If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. Do not induce vomiting. INHALATION: If inhaled, remove to fresh air. If breathing becomes difficult call a physician. **IF IN DOUBT SEEK MEDICAL ADVICE**
Fire fighting measures:	EXTINGUISHING MEDIA: Suitable: Carbon dioxide, Dry chemical powder or appropriate foam. SPECIAL RISKS: Emits toxic fumes under fire conditions. SPECIAL PROTECTIVE EQUIPMENT FOR FIREFIGHTERS: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Accidental release measures:	Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Place in covered containers. Avoid raising dust. Carefully sweep up and remove. Ventilate area and wash spill site after material pickup is complete.
Handling and storage:	Keep at –20^{o}C
Exposure Controls:	Wear appropriate protective clothing (safety spectacles, gloves, Laboratory coat) in accordance with Good Laboratory Practice.
Stability and reactivity:	Stable: Stable. Hazardous decomposition products: Nitrogen Oxides
Toxicological information:	Data not available
Ecological information:	Data not available
Disposal considerations:	Contact a licensed professional waste disposal service to dispose of this material.
Transport information:	Contact Ludger for transportation information.
Regulatory information:	Risk phrases: R36/37/38 Safety phrases: S26, 36
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.
DISCLAIMER:	For R&D use only. Not for drug, household or other uses.

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Abingdon 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 (2Molar) (Cat. No LT-ACETIC2M-01)
Composition:	Acetic acid in water (2 Molar). Ingredient Chemical name: Acetic Acid. CAS no. 64-19-7
Physical Properties:	MP/MP Range 16.2 °C, BP/BP Range 117 - 118 °C Colour: Colourless liquid.
Hazard identification:	Flammable. Causes severe burns.
First aid measures:	EYE CONTACT: In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. SKIN CONTACT: In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. INGESTION: If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. Do not induce vomiting. INHALATION: If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen. **IF IN DOUBT SEEK MEDICAL ADVICE**
Fire fighting measures:	EXTINGUISHING MEDIA: Suitable: Carbon dioxide, Dry chemical powder or appropriate foam. SPECIAL RISKS: Combustible liquid. Emits toxic fumes under fire conditions. SPECIAL PROTECTIVE EQUIPMENT FOR FIREFIGHTERS: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Accidental release measures:	Evacuate area. Shut off all sources of ignition. Use non-sparking tools. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Cover with dry-lime, sand, or soda ash. Place in covered containers using non-sparking tools and transport outdoors. Avoid raising dust. Carefully sweep up and remove. Ventilate area and wash spill site after material pickup is complete.
Handling and storage:	Store at room temperature away from sparks and open flames.
Exposure Controls:	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Stability and reactivity:	Stability: Stable. Avoid contact with oxidizing agents, carbonates, phosphates, hydroxides, oxides, metals, peroxides, permanganates, amines, alcohols.
Toxicological information:	Harmful if absorbed through skin, inhaled or if swallowed Material is extremely destructive to the tissue of the mucous membranes and upper respiratory tract.
Ecological information:	Data not available.
Disposal considerations:	Contact a licensed professional waste disposal service to dispose of this material.
Transport information:	Contact Ludger for transportation information.
Regulatory information:	Risk phrases: R10, 35 Safety phrases: S26, 36/37/39, 45
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.
DISCLAIMER:	For R&D use only. Not for drug, household or other uses.

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Abingdon 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:	Mercaptoethanol in Acetic Acid (Cat # LT-Mercapto-01)
Composition:	2-Mercaptoethanol (CAS no. 60-24-2) Acetic Acid (1.4 Molar) (CAS no. 64-19-7)
Physical Properties:	Colour: Colourless Liquid Odour: Stench
Hazard identification:	Harmful by inhalation and if swallowed. Toxic in contact with skin. Causes burns.
First aid measures:	EYE CONTACT: In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. SKIN CONTACT: In case of skin contact, flush with copious amounts of water for at least 15 minutes INGESTION: If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. Do not induce vomiting. INHALATION: If inhaled, remove to fresh air. If breathing becomes difficult call a physician. **IF IN DOUBT SEEK MEDICAL ADVICE**
Fire fighting measures:	EXTINGUISHING MEDIA Suitable: Water Spray, Carbon dioxide, Dry chemical powder or appropriate foam. SPECIAL RISKS Combustible liquid. Emits toxic fumes under fire conditions.

SPECIAL PROTECTIVE EQUIPMENT FOR FIREFIGHTERS
Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Accidental release measures: Evacuate area. Shut off all sources of ignition. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Place in covered containers. Absorb on sand or vermiculite and place in closed containers for disposal. Carefully sweep up and remove. Ventilate area and wash spill site after material pickup is complete.

Handling and storage: Keep at between 2 to 8^{o}C

Exposure Controls: Wear appropriate protective clothing (safety spectacles, gloves, Laboratory coat) in accordance with Good Laboratory Practice.

Stability and reactivity: Stable: Stable.
Avoid Heat and Moisture.
Avoid: Oxidizing agents, metals, carbonates, phosphates, hydroxides, oxides, peroxides, permanganates, amines and alcohols.

Toxicological information: Causes burns on skin contact
Toxic if absorbed through skin. Readily absorbed through skin.
Causes burns on eye contact
Harmful if inhaled. Material is extremely destructive to the tissue of the mucous membranes and upper respiratory tract.
Harmful if swallowed.

Ecological information: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Disposal considerations: Contact a licensed professional waste disposal service to dispose of this material.

Transport information: Contact Ludger for transportation information.

Regulatory information: Risk phrases: R10, 20/22, 24, 34, 35, 51/53
Safety phrases: S26, 36/37/39, 45, 61

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.
DISCLAIMER:	For R&D use only. Not for drug, household or other uses.

Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Abingdon 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:	Sodium Dithionite (Cat # LT-NaDithio-01)
Composition:	Sodium Dithionite CAS no. 7775-14-6
Physical Properties:	MP/MP Range 300 °C Colour: white powder
Hazard identification:	Flammable solid. Harmful if swallowed.
First aid measures:	EYE CONTACT: In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. SKIN CONTACT: In case of skin contact, flush with copious amounts of water for at least 15 minutes INGESTION: If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. Do not induce vomiting. INHALATION: If inhaled, remove to fresh air. If breathing becomes difficult call a physician. **IF IN DOUBT SEEK MEDICAL ADVICE**
Fire fighting measures:	EXTINGUISHING MEDIA Suitable: Dry chemical powder. Do NOT use water SPECIAL RISKS Flammable solid. Emits toxic fumes under fire conditions. SPECIAL PROTECTIVE EQUIPMENT FOR FIREFIGHTERS Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Accidental release measures:	Evacuate area. Shut off all sources of ignition. Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves. Place in covered containers. Avoid raising dust. Carefully sweep up and remove. Ventilate area and wash spill site after material pickup is complete.
Handling and storage:	Keep container closed. Keep away from heat, sparks, and open flames. Store in a cool dry place. Avoid contact with water Avoid contact with acid.
Exposure Controls:	Wear appropriate protective clothing (safety spectacles, gloves, Laboratory coat) in accordance with Good Laboratory Practice.
Stability and reactivity:	Stable: Stable. Avoid strong oxidizing agents and Acids Hazardous decomposition products: Sulfur oxides, Sodium/sodium oxides.
Toxicological information:	Data not available
Ecological information:	Data not available
Disposal considerations:	Contact a licensed professional waste disposal service to dispose of this material.
Transport information:	Contact Ludger for transportation information.
Regulatory information:	Risk phrases: R7, 22, 31 Safety phrases: S7/8, 26, 28, 43
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.
DISCLAIMER:	For R&D use only. Not for drug, household or other uses.

Ludger

Certificate of Conformity

LudgerTag Sialic Acid DMB Labeling Kit

Cat. # : LT-KDMB-A1 Lot # : A66C-02 Size : 1 sets of labeling reagents per kit

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This kit conforms to the specifications given in Ludger document # LT-KDMB-Ax-Guide .

Each kit contains the following components :

Quantity per Kit	Catalogue number	Lot number	Component Name
2	LT-ACETIC2M-01	A5CC-06	AceticAcid 2Molar
1	LT-MERCAPTO-01	A5CC-05	Mercaptoethanol
1	LT-DITHIO-01	A5CC-03	Sodium Dithioninte
1	LT-DMB-01	A5CC-04	DMB Dye
1	CM-SRP-01	A5AC-06	Sialic Acid Reference Panel
1	CM-NEUAC-01	A5CK-01	N-Acetyl Neuraminic acid
1	CM-NEUGC-01	A619-01	N-Glycolyl Neuraminic acid