



Product Guide for LudgerClean™ S

Glycan Cleanup Cartridges

(Ludger Product Code: LC-S-Ax, where x denotes pack size)

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Specifications for LudgerClean™ S Cartridges

Application For purification of glycans from a variety of complex mixtures including post-labeling cleanup of LudgerTag™ fluorophore and chromophore labeled glycans.

Description The cartridges contain a glycan binding membrane. This binds to glycans in solutions contain high levels of certain organic solvents (e.g. acetonitrile). Most hydrophobic non-glycan contaminants (e.g. aromatic dyes and detergents) either simply pass through the cartridges or bind very lightly and can be washed off the membrane. The glycans are eluted from the membrane with water.



Number of Samples LudgerClean™ S cartridges are designed for single use only.

Amount of Sample Each cartridge can, under typical conditions, bind up to 20 µg of glycan.

Suitable Samples A wide range of glycans can be purified. These include N-linked and O-linked type oligosaccharides, tri-saccharides and larger structures.
The cartridges are **not** generally suitable for monosaccharides or disaccharides which are generally bound too weakly for efficient purification.

Structural Integrity No detectable (< 2 mole per cent) loss of sialic acid, fucose, sulfate, or phosphate.

Binding efficiency > 90 % for most suitable glycans.

Binding Selectivity Essentially stoichiometric binding and elution for most complex glycan mixtures.

Storage: Store at room temperature in the dark. Protect from sources of heat, light, and moisture. The cartridges 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.
- Safety:** Please read the Material Safety Data Sheets (MSDS's) for all chemicals used. All processes involving hazardous 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

Additional Reagents and Equipment Required

- Pure water, HPLC grade (~ 3 ml per sample)
- Acetonitrile, HPLC grade (~ 3 ml per sample)
- 96 % acetonitrile / 4 % water (v/v) (~ 6 ml per sample)
- 30 % acetic acid / 70 % water (v/v) (~ 6 ml per sample)

Introduction

LudgerClean™ S cartridges have been designed for purification of glycans from non-carbohydrate material including salts, proteins, and detergents. Applications include cleanup of glycans following hydrazinolysis, endoglycosidase digests (including PNGase F digests), and enzyme treatment, and before and after fluorescent labeling.

Outline of LudgerClean™ S Cleanup Protocol

The outline of the cleanup procedure is as follows :

1 Prime the membrane

The active surface of the glycan binding membrane is primed by washing with water, 30% acetic acid, then acetonitrile.

2 Apply the glycan sample

The glycan sample (maximum volume 15 µl) is spotted onto the membrane.

3 Wash off the non-glycan contaminants

Non-glycan contaminants such as detergents and fluorescent dye are washed out using wash solutions containing high concentrations of acetonitrile.

4 Elute the glycans

Bound glycans are washed off the cartridge with water.

5 Post elution workup

The eluted glycan solution is filtered.

6 Analyse the glycans

The glycans are now ready for analysis.

Time Line for Cleanup

The LudgerClean™ S glycan cleanup procedure typically takes around 80 - 100 minutes :

Procedure	Time	Elapsed Time (minutes)
Wash and prime cartridges	20 min	20
Apply sample	20 min	40
Wash off non-glycan contaminants	20 min	60
Elute glycans	20 min	80

Instructions for Use

1 Prepare the glycan sample

The sample to be cleaned must have a volume of 15 µl or less. If your sample has a greater volume then dry it down using a centrifugal evaporator and reconstitute in not more than 15 µl of water

Preparation of Cartridges

2 Prime the LudgerClean™ S cartridge

Prime the LudgerClean™ S cartridges (one per sample) by washing each with the following :

- 1 ml water.
- 5 ml 30 % acetic acid (aq).
- 1 ml acetonitrile.

Allow each wash to drain completely before adding the next. If flow is restricted, e.g. by an air gap, apply a slight pressure to the top of the cartridge in order to resume normal flow.

Application of Sample and Removal of Contaminants

3 Spot sample onto cartridge membrane

Spot each sample onto a freshly washed cartridge disc ensuring that the disc is still wet with acetonitrile.

If a disc has dried out it can be washed with a further 0.5 ml acetonitrile prior to loading the sample. When applying the sample to the cartridge, spread the spot over the entire centre of the adsorption disc - this will give a more efficient cleanup than a small spot.

4 Allow sample to adsorb onto membrane

Leave for 15 minutes while the glycans adsorb onto the disc.

5 Add residual sample from sample vial

Rinse each sample vial with 100 µl acetonitrile and apply to the corresponding cartridge disc.

6 Wash non-glycan contaminants off membrane

Wash each disc with 1 ml acetonitrile, followed by 5 x 1 ml 96 % acetonitrile / 4% water.

Discard these washes into a suitable waste container.

Elution of Glycan

7 Elute glycans off membrane

Recover the glycans by eluting with 3 x 0.5 ml water.

Allow each 0.5 ml aliquot to drain before the next is applied.

8 Dry the eluted glycans (optional)

If appropriate, evaporate the glycan containing fraction to dryness, then redissolve in a desired volume of

water or solvent for further analysis.

9 Filter the eluted glycans

Samples should be filtered to at least 0.5 µm.

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

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Material Safety Data Sheet

Manufacturer	Ludger Ltd Culham Science Centre, Oxford OX14 3EB, UK Tel: +44 870 085 7011, Fax: +44 870 163 4620 Email: safety@ludger.com, Website: www.ludger.com
Identification of the substance	LudgerClean™ S cartridges
Composition	Tube of polypropylene containing a glycan absorption disc
Hazard identification	Non hazardous.
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 at room temperature. Handle in accordance with Good Laboratory Practice.
Exposure Controls /	Wear appropriate protective clothing (safety spectacles, gloves, laboratory coat) in accordance with Good Laboratory Practice.
Physical and chemical properties	Constructed of solid plastic and polymeric materials
Stability and reactivity	Not combustible.
Toxicological 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.
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.