



Certificate of Analysis

LudgerZyme™ Ceramide Glycanase Kit

Cat. #: LZ-CER-HM-KIT

Batch: C01G-04

Size: 1 set of enzyme per kit

Expiry date: 16 Jan 2022

This kit conforms to the specifications given in Ludger document # LZ-CER-HM-KIT-Guide-v1.0

Each kit contains the following components:

Quantity per Kit	Cat #	Batch #	Component Name
1	LZ-CER-HM-10	C01G-03	LudgerZyme Ceramide Glycanase (<i>Hirudo medicinalis</i>)
1	LZ-CER-BUFFX4	C01G-02	LudgerZyme Ceramide Glycanase RXN buffer
1	GLIP-GM1-01	C01G-01	GM1 glycolipid (positive control)

Ceramide glycanase assay: 5µL of 20µU/mL LudgerZyme Ceramide Glycanase (LZ-CER-HM-10) were incubated with 5µL of 0.33µg/µL GM1 glycolipid (GLIP-GM1-01), in a 20µL reaction containing 5µL 3X LudgerZyme Ceramide Glycanase RXN buffer (LZ-CER-BUFFX3). Reaction was incubated for 24 hours at 37°C. The reaction products were cleaned up by filtering through protein binding membrane (LC-PBM-96). The released glycans were 2AB labelled (LT-KAB-A2) and S-cartridge clean-up (LC-S-A6). 2AB labelled products were analysed by HILIC-HPLC.

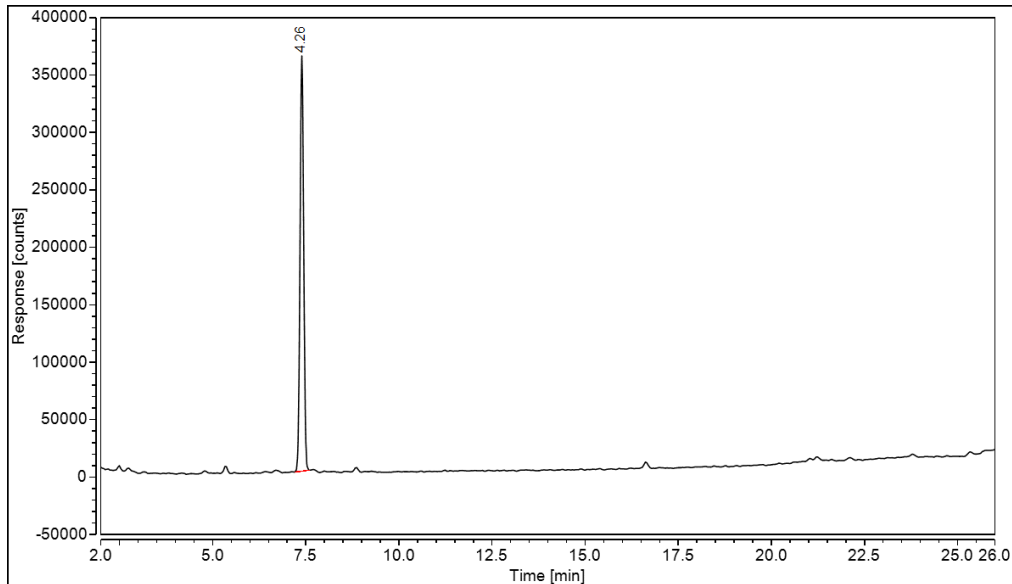


Figure 1: HILIC HPLC profile of two 2AB labelled GLIP-GM1-01 glycans (Batch: C01G-01) released using LZ-CER-HM-KIT (Batch: C01G-04)

HPLC Running Conditions:

Column: Waters BEH Glycan 1.7µm column (150mm)

Flow: 0.56mL/min.

Temperature: 60 °C

Solvent A: 50mM ammonium formate pH 4.4 **Solvent B:** 100 % acetonitrile

Gradient:

Time (min)	%B
0.0	78.0
1.5	78.0
24.8	58.0
25.8	40.0
25.9	78.0
31.0	78.0

Detector: Fluorescence

Excitation wavelength: 250 nm

Emission wavelength: 428 nm

No contaminating exoglycosidase or endoglycosidase activities were detected (ND) with the following substrates:

α -sialidase and endoglycosidase F3:

2AB-FA2G2S2 (CAB-A2F-01)

ND

β -galactosidase and α -fucosidase:

2AB-FA2G2 (CAB-NA2F-01)

ND

β -glucosaminidase and α -fucosidase:

2AB-FA2 (CAB-NGA2F-01)

ND

α -mannosidase and endoglycosidase F1 & F2:

2AB-mannose (CAB-MAN5-01, CAB-MAN6-01, CAB-MAN7-01, CAB-MAN8-01 and CAB-MAN9-01)

ND

α -galactosidase:

2AB B2 trisaccharide

ND