



Certificate of Analysis

LudgerZyme Ceramide Glycanase Kit

Cat. #: LZ-CER-HM-KIT

Batch #: B6B7-01

Size: 1 set of enzyme per kit

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	B6B1-02	LudgerZyme Ceramide Glycanase (<i>Hirudo medicinalis</i>)
1	LZ-CER-BUFFX4	B6B1-01	LudgerZyme Ceramide Glycanase RXN buffer
1	GLIP-GM1-01	B6B1-03	GM1 glycolipid (positive control)

Expiry Date: November 2018



Ceramide glycanase assay: 5 μ L of 20 μ U/mL LudgerZyme Ceramide Glycanase (LZ-CER-HM-10) were incubated with 5 μ L of 0.33 μ g/ μ L GM1 glycolipid (GLIP-GM1-01), in a 20 μ L reaction containing 5 μ L 4X LudgerZyme Ceramide Glycanase RXN buffer (LZ-CER-BUFFX4). Reaction was incubated for 24 hours at 37°C. 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.

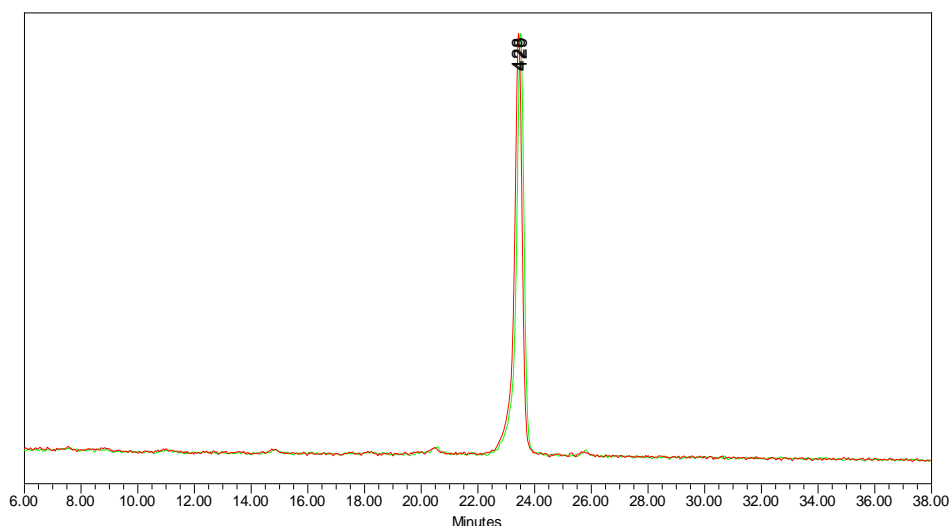


Figure 1: HILIC HPLC profile of two 2AB labelled GLIP-GM1-01 glycans (Batch: B6B1-03) released using LZ-CER-HM-KIT (Batch: B6B7-01)

HPLC Running Conditions:

Column: LudgerSep-N2 (LS-N2-4.6x150)

Solvent A: 50 mM ammonium formate pH 4.4 Solvent B: 100 % acetonitrile Temperature: 35 °C.

Gradient: 0 min – 22% A, Flow 0.56 ml/min; 1.5 min – 22%; 24.8 min – 42% A; 25.8 min – 60% A; 25.9 min – 100% A, Flow 0.25ml/min; 27.9 min – 100%; 28.4 min – 22%; 32 min – 22%, Flow 0.56ml/min; 35 min – 22%.

Detector: Waters 2475 FLR Detector Excitation wavelength: 330 nm Emission wavelength: 420 nm

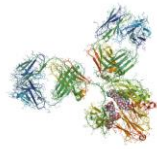
No contaminating exoglycosidase or endoglycosidase activities were detected (ND) with the following substrates:

α -sialidase and endoglycosidase F3:
2AB-FA2G2S2 (CAB-A2F-01)

ND

β -galactosidase and α -fucosidase:
2AB-FA2G2 (CAB-NA2F-01)

ND



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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