Certificate of Analysis

LudgerZyme™ Ceramide Glycanase Kit

Cat. #: LZ-CER-HM-KIT
Batch: B95G-03
Size: 1 set of enzyme per kit
Expiry date: 16 May 2021

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	B95E-02	LudgerZyme Ceramide Glycanase (*Hirudo medicinalis*)
1	LZ-CER-BUFFX4	B95E-01	LudgerZyme Ceramide Glycanase RXN buffer
1	GLIP-GM1-01	B95E-03	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.

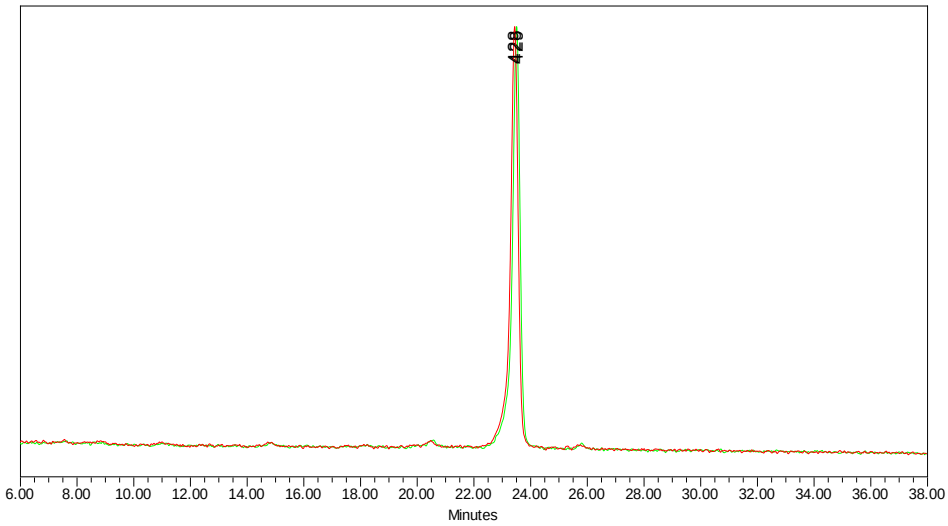

***Figure 1**: HILIC HPLC profile of two 2AB labelled GLIP-GM1-01 glycans (Batch: B54N-02) released using LZ-CER-HM-KIT (Batch: B54O-02)*

HPLC Running Conditions:

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

Flow: 1.0 mL/min

Solvent A: 50 mM ammonium formate pH 4.4

Solvent B: 100 % acetonitrile

Temperature: 35 °C.

Gradient: 0 min – 20% A, 36 min – 38% A, 36.5 min – 100% A, 38.5 min – 100% A, 40 min – 20% A, 45 min – 20%

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

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