Certificate of Analysis

LudgerZyme Ceramide Glycanase Kit

Cat. #: LZ-CER-HM-KIT | Batch #: B54O-02 | 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	B54M-05	LudgerZyme Ceramide Glycanase *(Hirudo medicinalis)*
1	LZ-CER-BUFFX3	B54M-04	LudgerZyme Ceramide Glycanase RXN buffer
1	GLIP-GM1-01	B54N-02	GM1 glycolipid (positive control)

Expiry Date: Jan 2017

Ceramide glycanase assay: 2µL of 20µU/mL LudgerZyme Ceramide Glycanase (LZ-CER-HM-10) was 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 released glycans were 2AB labelled (LT-KAB-A2) and S-cartridge clean-up (LC-S-A6). 2AB labelled products were analysed by HILIC-HPLC.

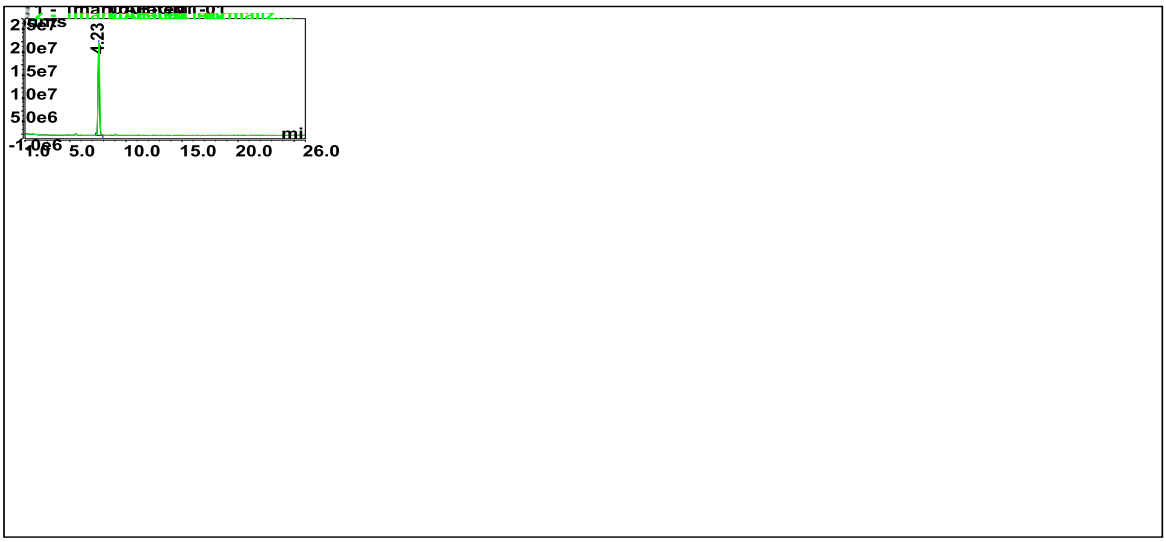

Figure 1: HPLC profile of two 2AB labelled GLIP-GM1-01 glycans (Batch: B54N-02) released using LZ-CER-HM-KIT (Batch: B54O-02)

HPLC Running Conditions:

Column: BEH Flow: 0.56 mL/min

Solvent A: 50 mM ammonium formate pH 4.4 Solvent B: 100 % acetonitrile Temperature: 60 °C.

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: U3000 FLD 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