Certificate of Stability

LZ-PNGaseL-50-KIT

Stability Question

What is the enzyme LZ-PNGaseL-01-50 (Batch #) performance when exposed to higher ambient temperatures for a prolonged period of time?

Stability Assay

LZ-PNGaseL-50-KIT was subjected to 37˚C, room temperature or 4°C for up to 15 days. Each kit was then used for the N-glycan release of Horse radish peroxidase (HRP) and Human IgG glycoprotein. For HRP a stock solution of 1.1 mg/mL was prepared using Horse radish peroxidase from sigma (P6782-10MG), 10 µg were used for each release. For IgG GCP-IgG-50 (Batch# C14C-01) was used.Glycoproteins were denatured with 1µL LudgerZyme Denaturation Solution-2, incubation for 5-10 min at 100 ^{o}C. After cooling down the sample, 2 µL of LudgerZyme NP-40 10% solution-2, 2 µL of LudgerZyme 10X-2 Reaction Buffer solution, 4 µL of water and 2 µL of LudgerZyme PNGase L enzyme were added and incubated at 37°C for 1 hour. Reaction products were cleaned up by filtering through protein binding membrane (LC-PBM-96). The released glycans were PROC labelled (LT-KPROC-24) and cleaned up (LC-PROC-96). PROC labelled products were analysed by HILIC UHPLC.

Stability Outcome.

LZ- PNGaseL-50-KIT remains active against HRP after 7 days incubation at either 37˚C, room temperature or at 4°C (Figure 1)

LZ- PNGaseL-50-KIT remains active against HRP after 15 days incubation at either 37˚C, room temperature or at 4°C (Figure 2).

LZ- PNGaseL-50-KIT remains active against human gamma globulins after 7 days incubation at either 37˚C, room temperature or at 4°C (Figure 3).

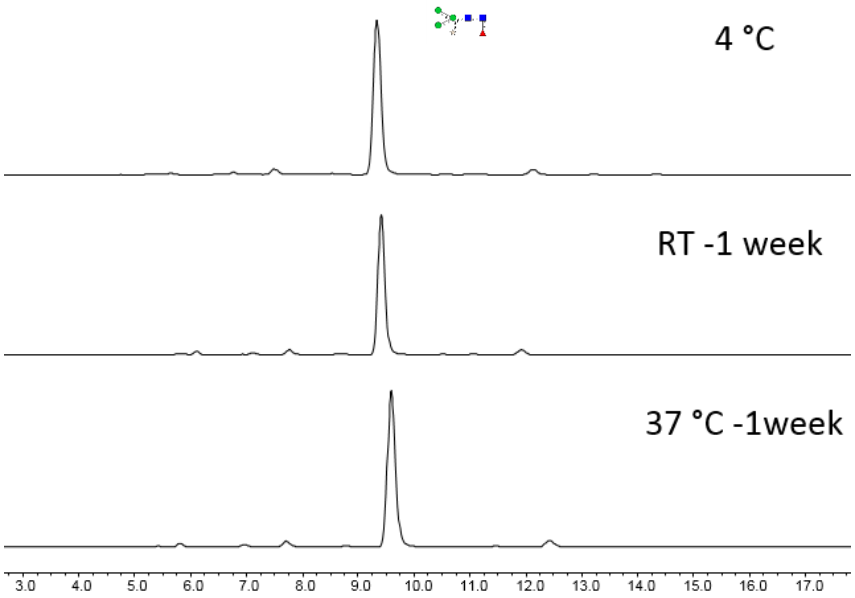


Figure 1. HILIC-UHPLC stack profiles of procainamide labelled HRP N- glycan released with **LZ- PNGaseL-50-KIT** , following enzyme storage under stress conditions (room temperature and 37°C) for 1 week. Y axis normalised.

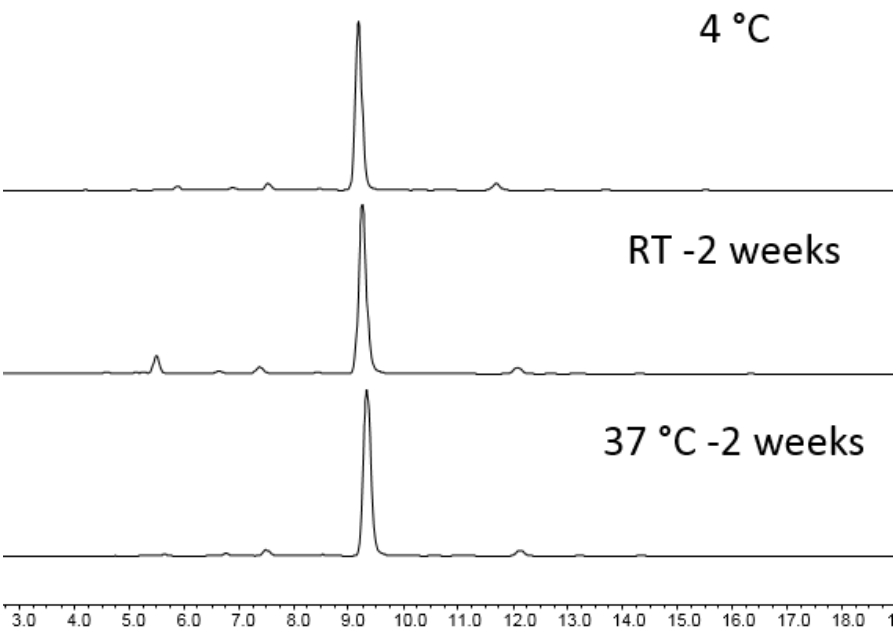


Figure 2. HILIC-UHPLC stack profiles of procainamide labelled HRP N- glycan released with **LZ- PNGaseL-50-KIT** , following enzyme storage under stress conditions (room temperature and 37°C) for 2 week. **Y axis normalised.**

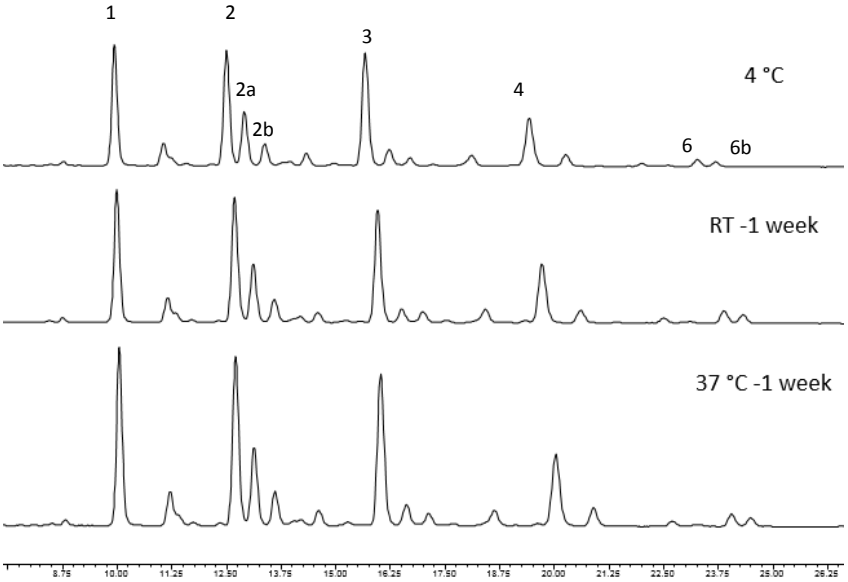
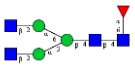
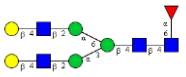
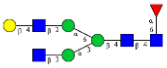
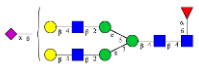
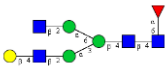
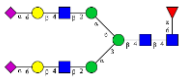
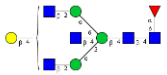


Figure 3. *HILIC-UHPLC stack profiles of procainamide labelled IgG N- glycan released with* ***LZ- PNGaseL-50-KIT*** *, following enzyme storage under stress conditions (room temperature and 37°C)* for 1 week. ***Y axis normalised.***

Peak	Glycan Name	Structure	Peak	Glycan Name	Structure
1	NGA2F		3	NA2F	
2	FA2[6]G1		4	A1F	
2a	FA2[3]G1		6	A2F	
2b	FA2[6]BG1		6b	A2FB