Antibody Validation Report

aVR.CXCL8.P10145.AB_2609541.v1.0 (March 25_2021)

A. Basic Target Information

Target Information

UniProt Accession Number: P10145

Target Name: Interleukin-8

Antibody Information

RRID: AB_2609541

Antibody Name: IL-8 (CXCL8) Monoclonal Antibody (6217)
Host Organism: Mouse

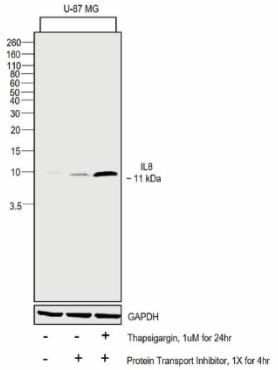
Clonality: Monoclonal
Vendor: Thermo
Catalog Number: MA5-23697
Lot Number: WA3179776
Recombinant (Y/N): No

Organ/Tissue used for validation: THP-1 cells
HuBMAP Platform Used: IP-MS

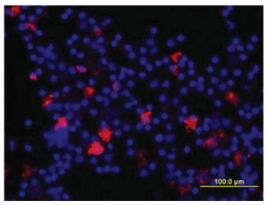
Protocols.io doi for Validation Protocol: 10.17504/protocols.io.btztnp6n
ORCID ID of submitter: 0000-0002-5631-512X, 0000-0002-6095-2797

B. Validation Data

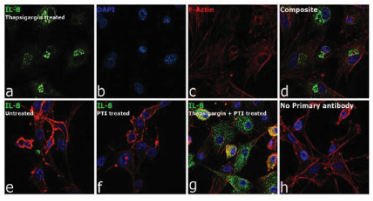
B.1. Vendor Validation: WB, IF, IHC, FC



Lane 1: U-87 MG
Lane 2: U-87 MG treated with PTI (1x 4hr)
Lane 3: U-87 MG treated with Thapsigargin (1uM for 24hr) followed by PTI (1X for 4hr)
Primary: Anti-IL-8 (CXCL8) Monoclonal Antibody (6217)(MA5-23697) (1 µg/mL) Secondary: Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (A28177, 1:4000 dilution)



Human peripheral blood mononuclear cells (PBMCs)
Primary: CXCL8/IL-8 Monoclonal Antibody (MA5-23697) at 10 µg/mL for 3 hours at room temperature.
Secondary: 557-conjugated Anti-mouse IgG Secondary Antibody (re and counterstained with DAPI (blue).



I U-87 MG cells treated with 1uM of Thapsigargin for 24 hours.
Panel a: IL-8 (green)
Primary: IL-8 (CXCL8) Monoclonal Antibody (6217) (MA5-23697) at 8 µg/mL in 0.1% BSA, 4C overnight
Secondary: Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (P# A32723), (1:2000), 45 minutes at room temperature
Panel b: nuclei- ProLong™ Diamond Antifade Mountant with DAPI (P36962).
Panel c: F- Actin-Rhodamine Phalloidin (R415, 1:300).
Panel d: merged image
Panel e: untreated cells
Panel f: cells treated with PTI
Panel g: cells treated with Thapsigargin and PTI
Panel h:control cells with no primary antibody

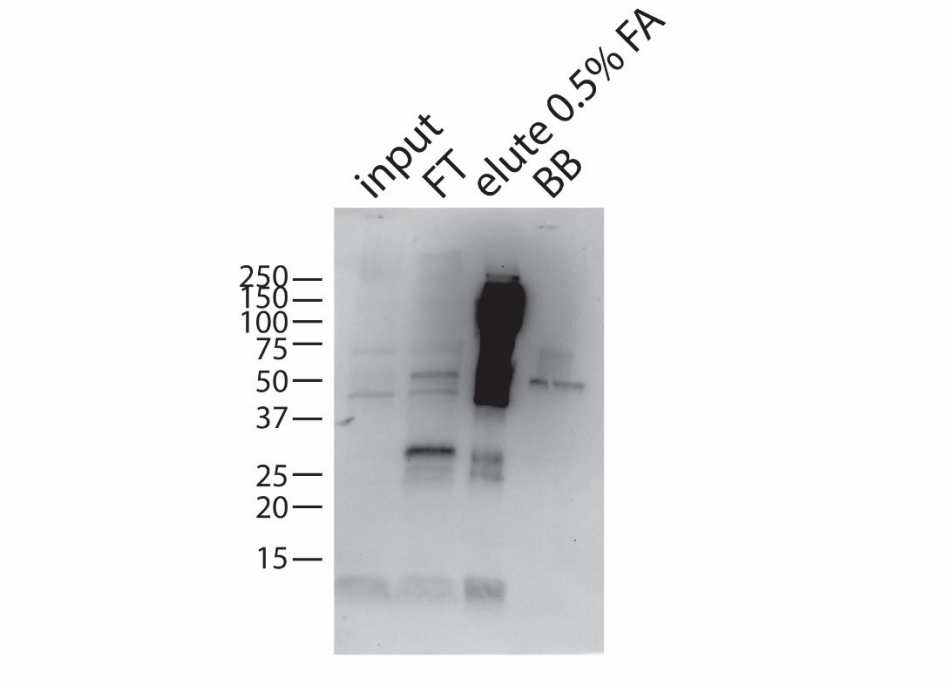
Date Accessed: 03/25/21

URL: https://www.thermofisher.com/antibody/product/IL-8-CXCL8-Antibody-clone-6217-Monoclonal/MA5-23697

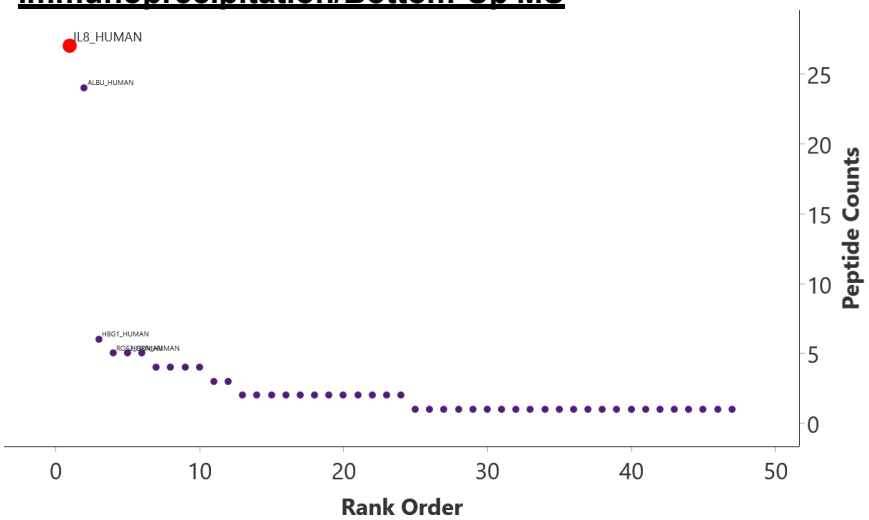
B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

Primary: anti-IL-8 (3IL8-H10) (ThermoFisher M801_AB_223583), 1:2000 on 4C
Secondary Secondary goat anti-mouse (Abcam ab6789), 1/5000 1 h RT



Immunoprecipitation/Bottom-Up MS



Immunoprecipitation/Top-Down MS

Proteoforms Identified:
1: PFR00000072722, Interleukin-8, 8916.74 Da
AVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQRV
VEKFLKRAENS

2: PFR00000005811, MDNCF-a, 9102.81 Da
EGAVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQ
RVVEKFLKRAENS

3: PFR00000227828, Interleukin-8 (9-77)
ELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQRVVEKFLKRA
ENS

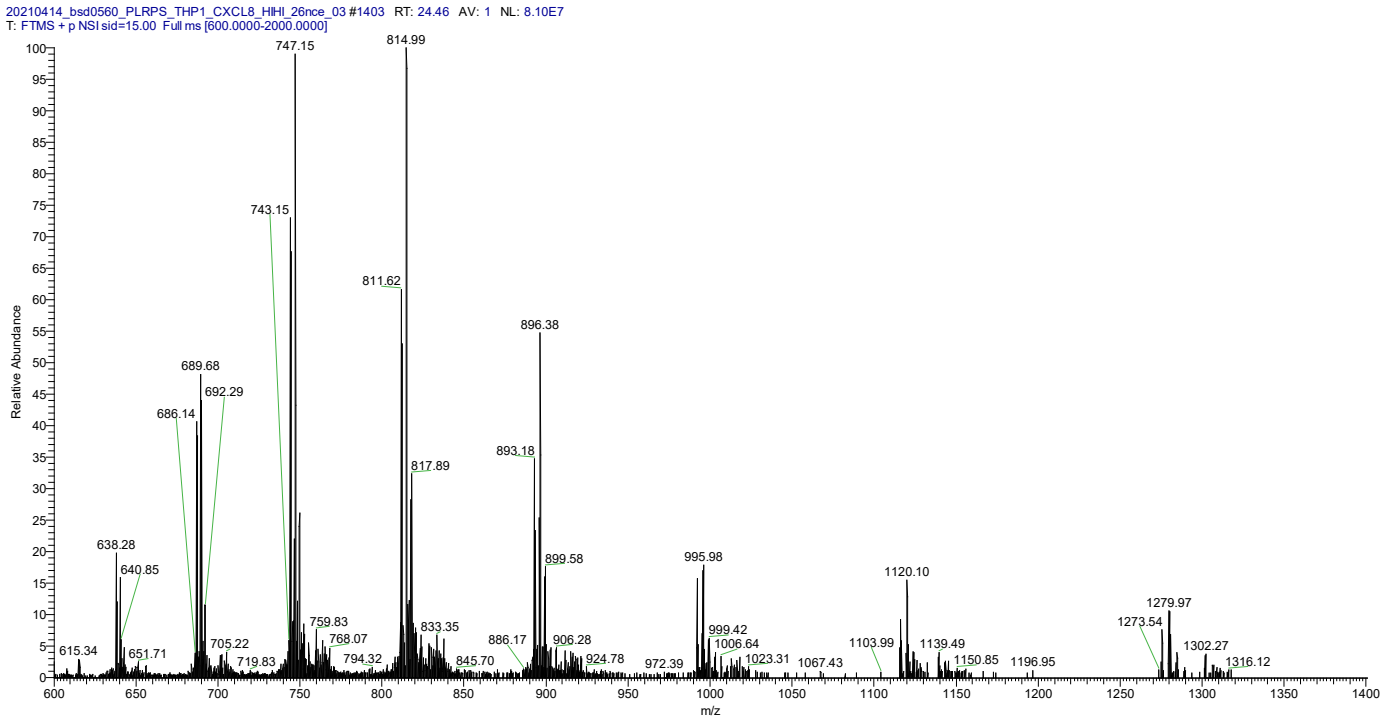
4: PFR00005931242, Interleukin-8 (1-57), 6,478.43 Da
AVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLD

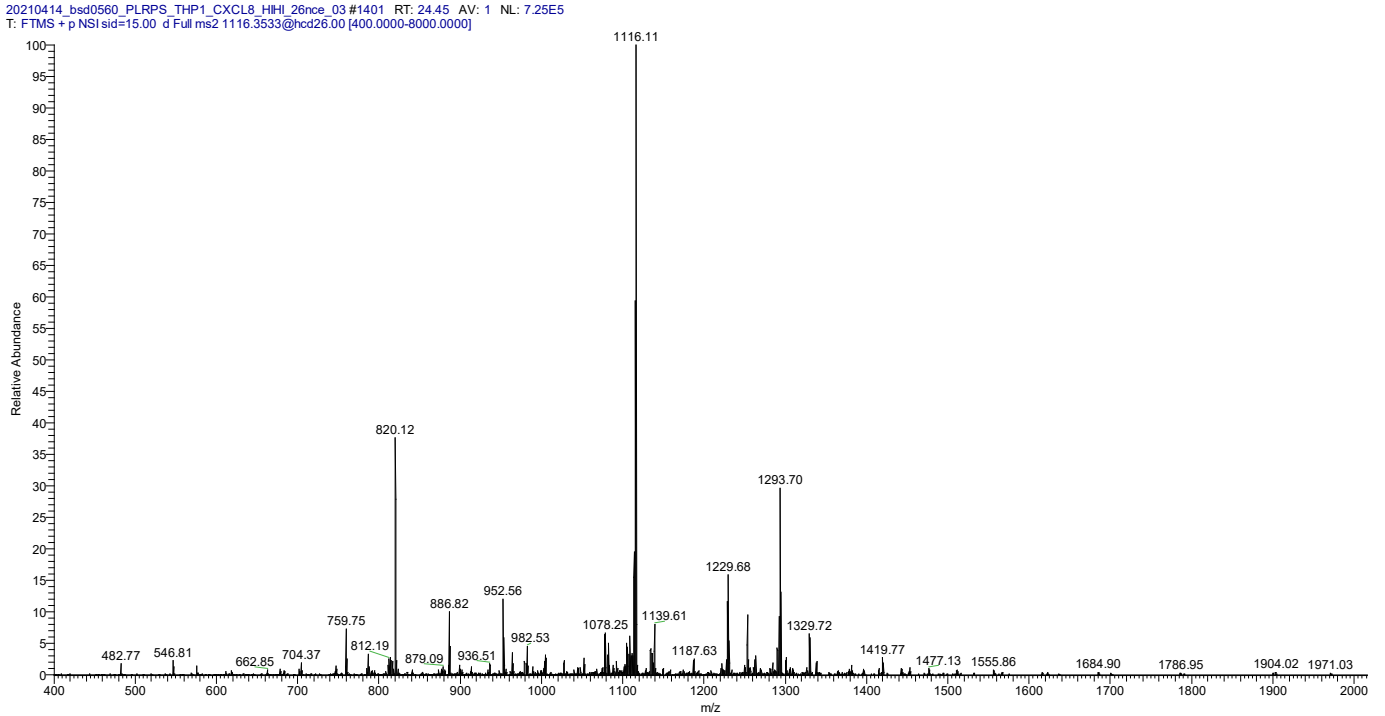
5: PFR00005931243, MDNCF-a (1-59): 6,664.49 Da
EGAVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSDGRELCLD

6: PFR00005931244, Interleukin-8 (1-50): 56838
AVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSD

Intact Mass Profile

PFR00000072722:





N A V L P R S A K E L R C Q C I K T Y S K P F H P K 25

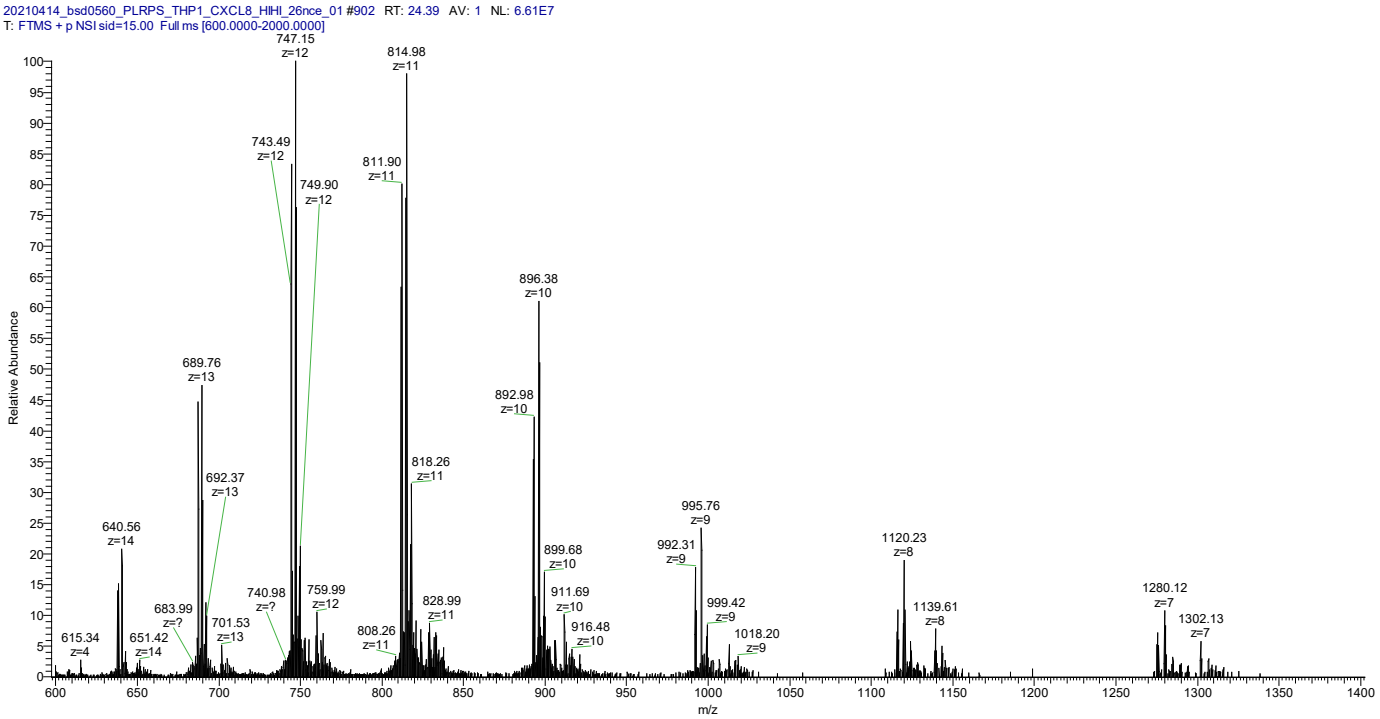
26 F I K E L R V I E S G P H C A N T E I I V K L S D 50

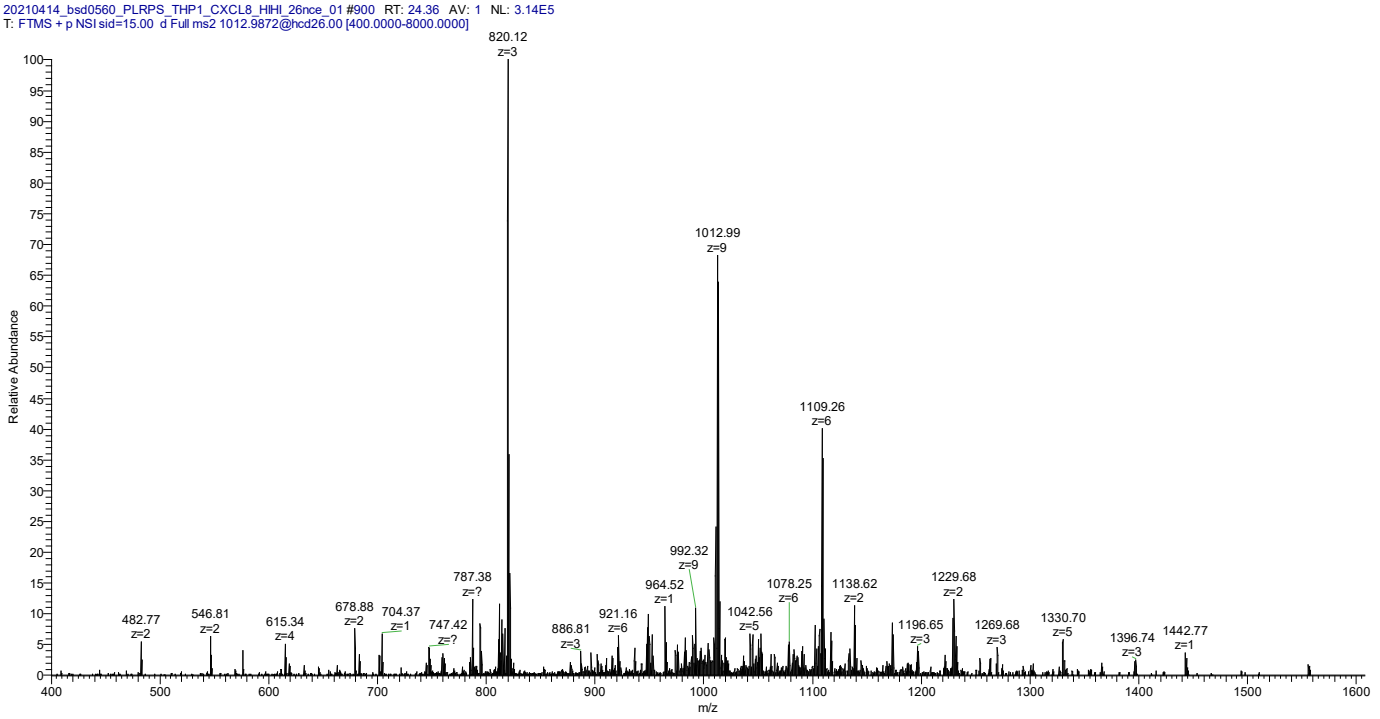
51 G R E L C L D P K E N W V Q R V V E K F L K R A E 75

76 N S C

Observed: 8,916.76
Theoretical: 8,916.74
Mass Diff. (Da): 0.016
Mass Diff. (ppm): 1.83
P-score: 4.6e-89

PFR00000005811:





N E G A V L P R S A K E[L R]C Q C[I]K T Y]S]K[P F]H 25

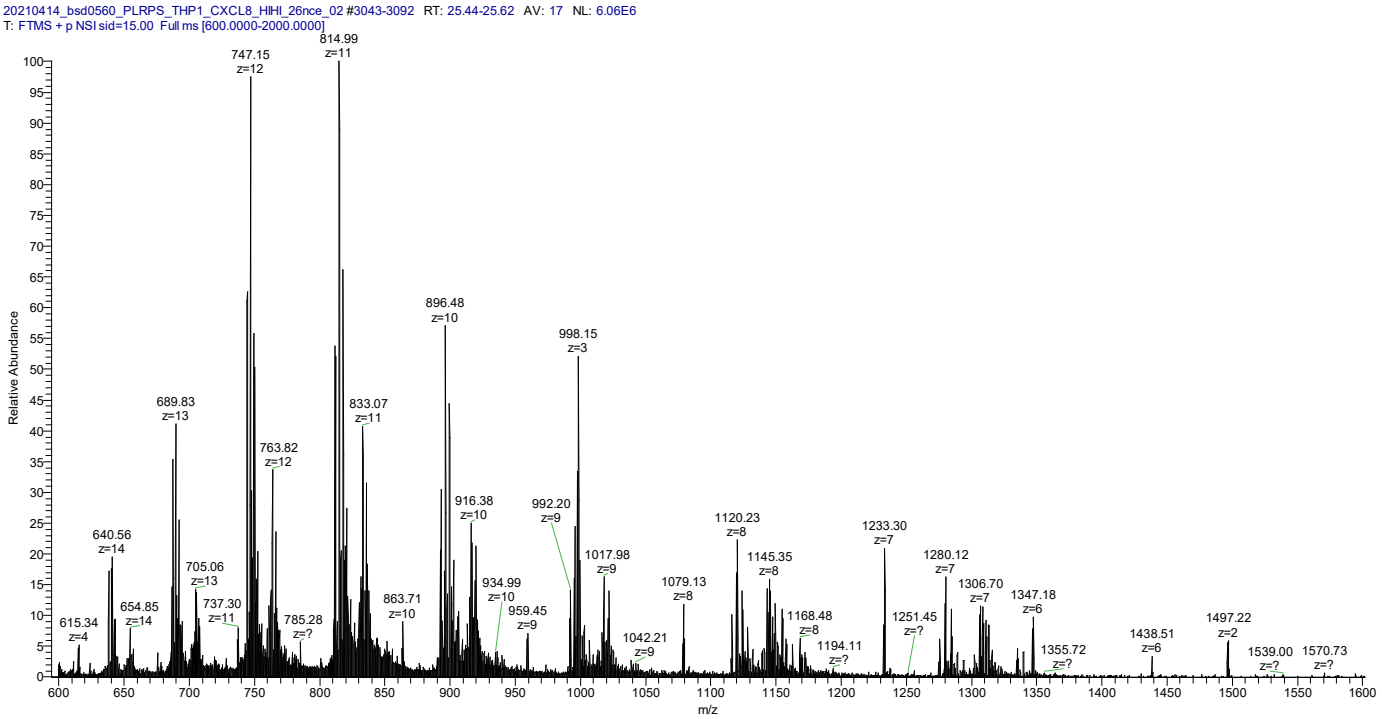
26[P K F I K E[L R V I E]S G]P H[C]A]N]T[E]I]I[V]K[L 50

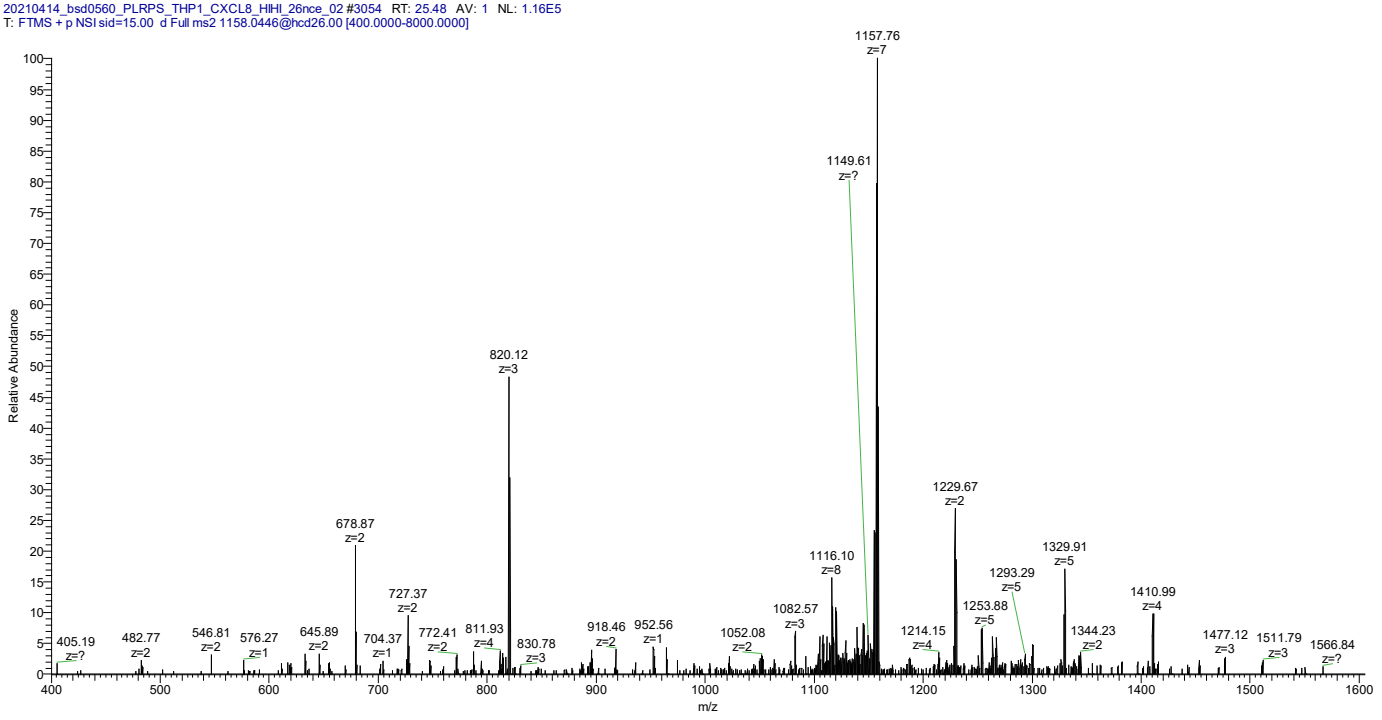
51 S D[G R E L C]L D[P[K[E[N W[V]Q R V]V[E]K[F]L]K]R 75

76 A E N S C

Observed: 9,102.82
Theoretical: 9,102.81
Mass Diff. (Da): 0.006
Mass Diff. (ppm): 0.68
P-score: 6.6e-70

PFR00000227828:





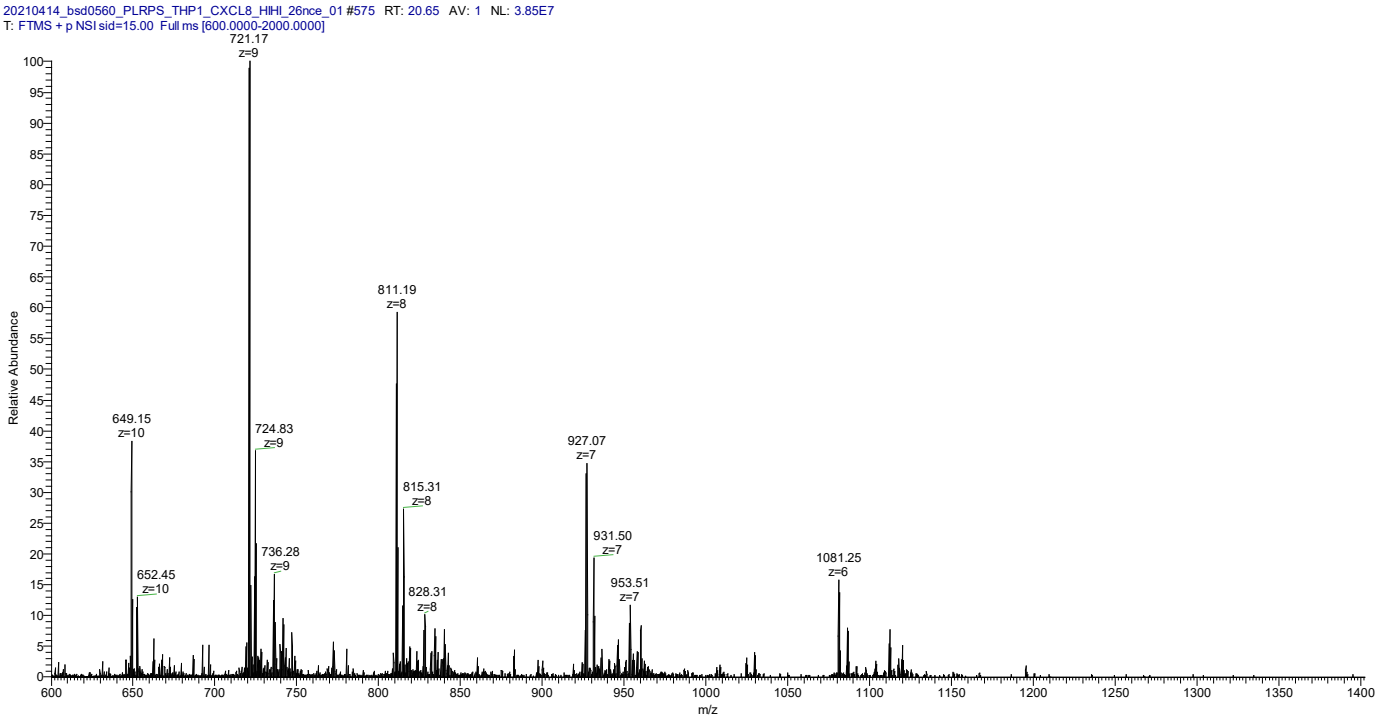
N E L L R C Q C I K T Y S K P F H P K F I K E L R V I 25

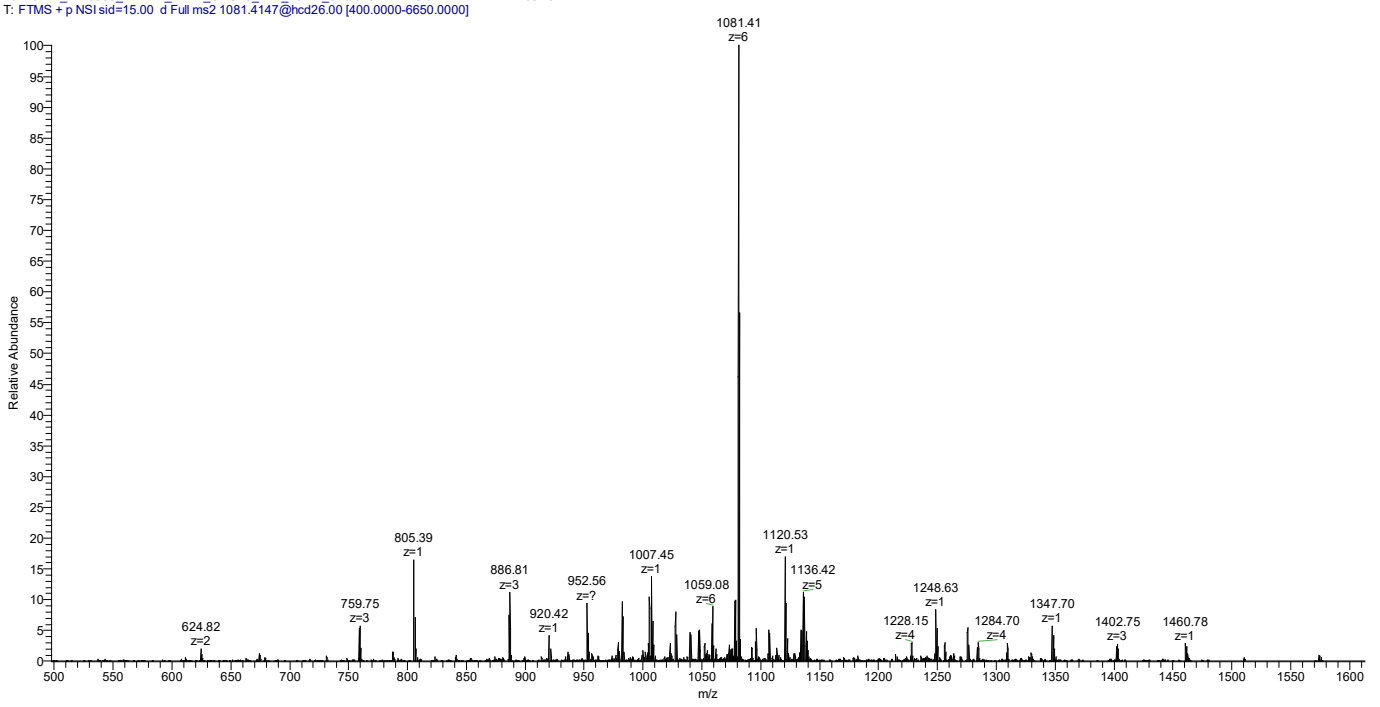
26 E S G P H C A N T E I I V K L S D G R E L C L D P 50

51 K E N W V Q R V V E K F L K R A E N S C

Observed: 8,094.26
Theoretical: 8,094.24
Mass Diff. (Da): 0.021
Mass Diff. (ppm): 2.6
P-score: 1.4e-31

PFR00005931242:





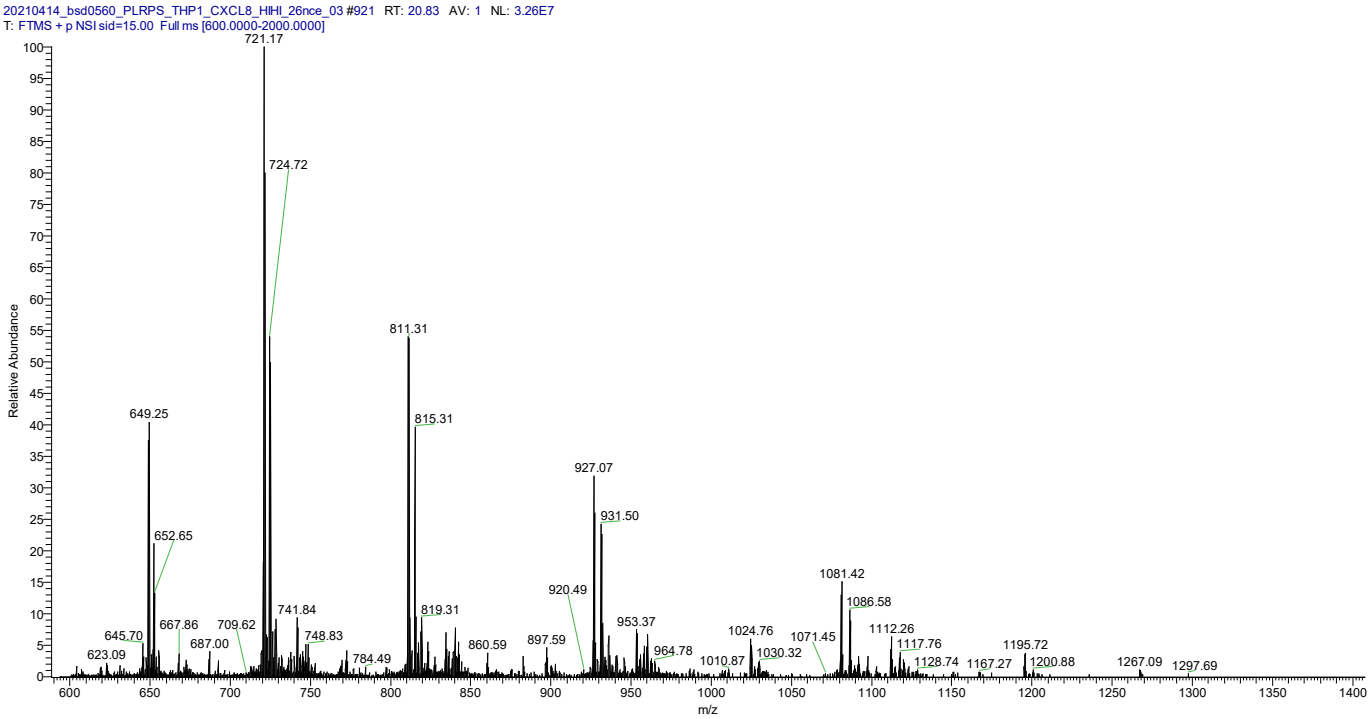
N A V L P R S A K[E]L R[C]Q[C] I K T Y[S]K[P]F[H]P[K] 25

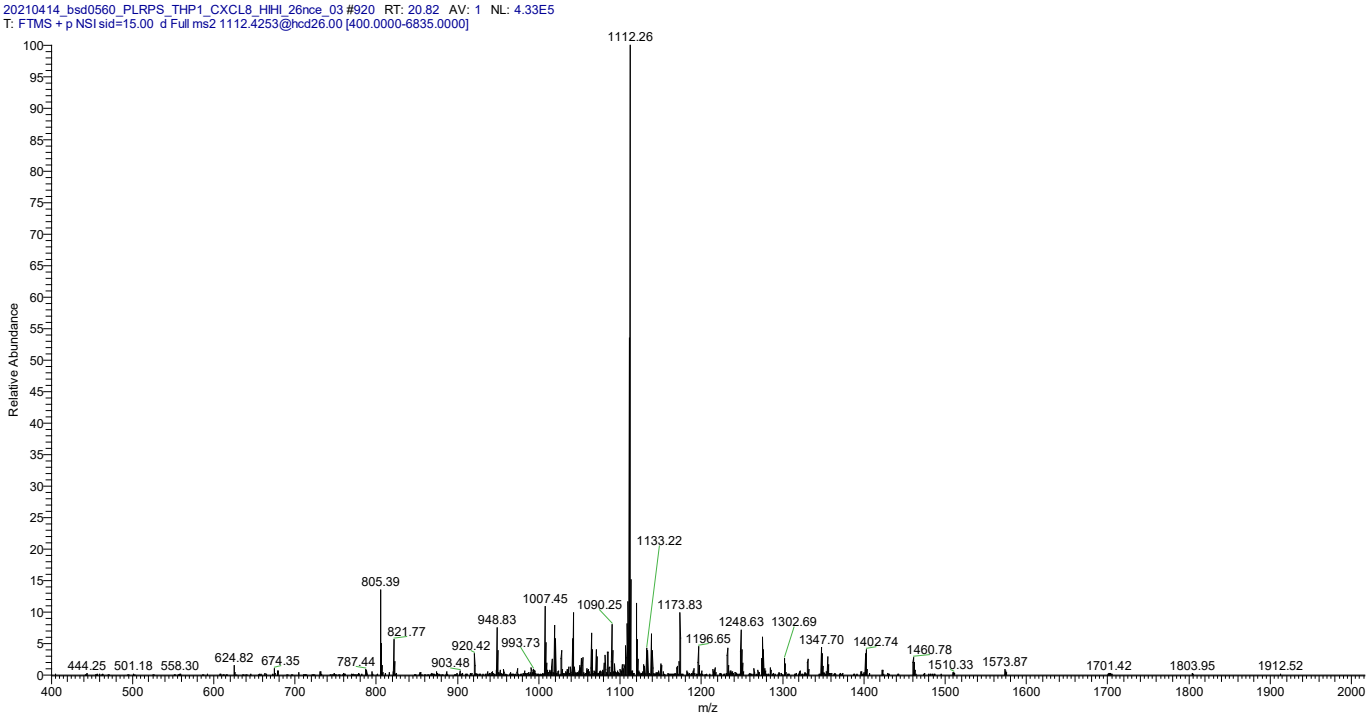
26[F] I K E[L] R[V] I[E]S G P H[C]A[N]T[E] I[I]V[K]L[S]D 50

51[G] R[E]L[C]L[D] C

Observed: 6,478.44
Theoretical: 6,478.43
Mass Diff. (Da): 0.017
Mass Diff. (ppm): 2.6
P-score: 4.4e-97

PFR00005931243:





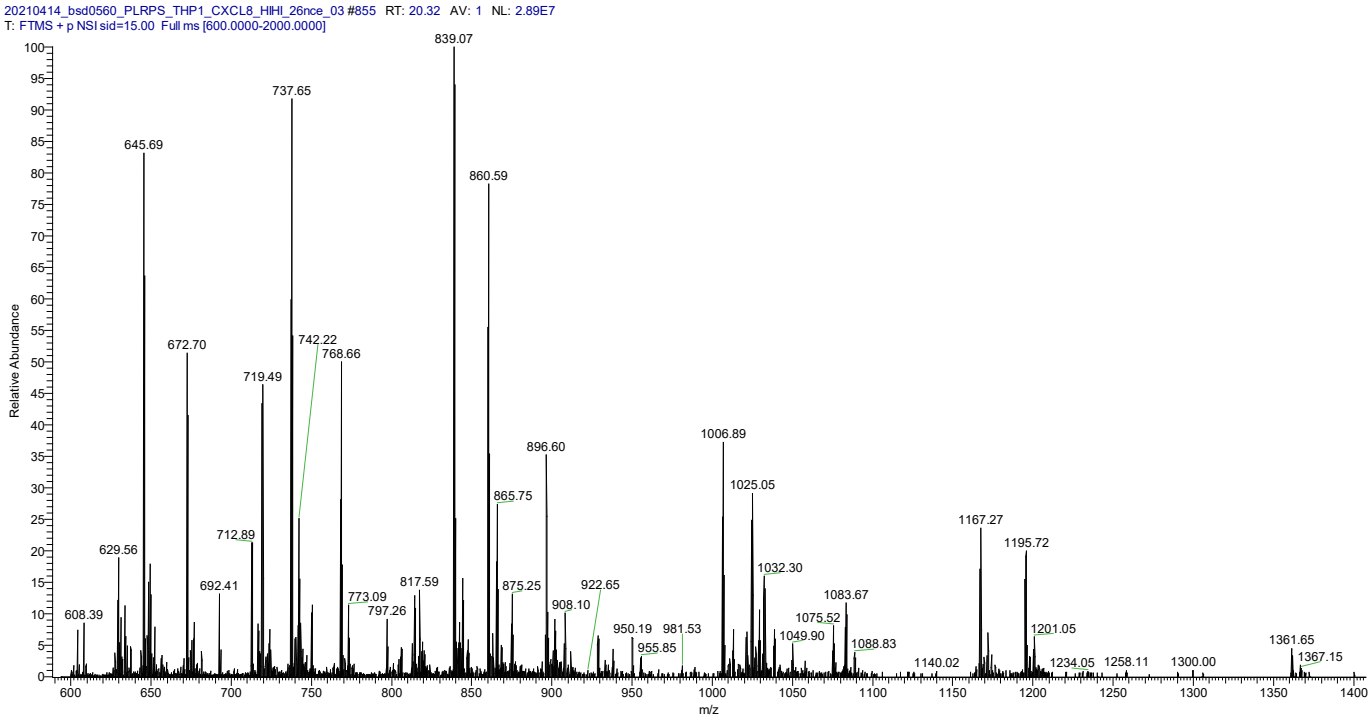
N E G ⌊A V L P R S A K E⌋L R⌉C Q C I K⌉T Y S K⌊P⌉F⌊H 25

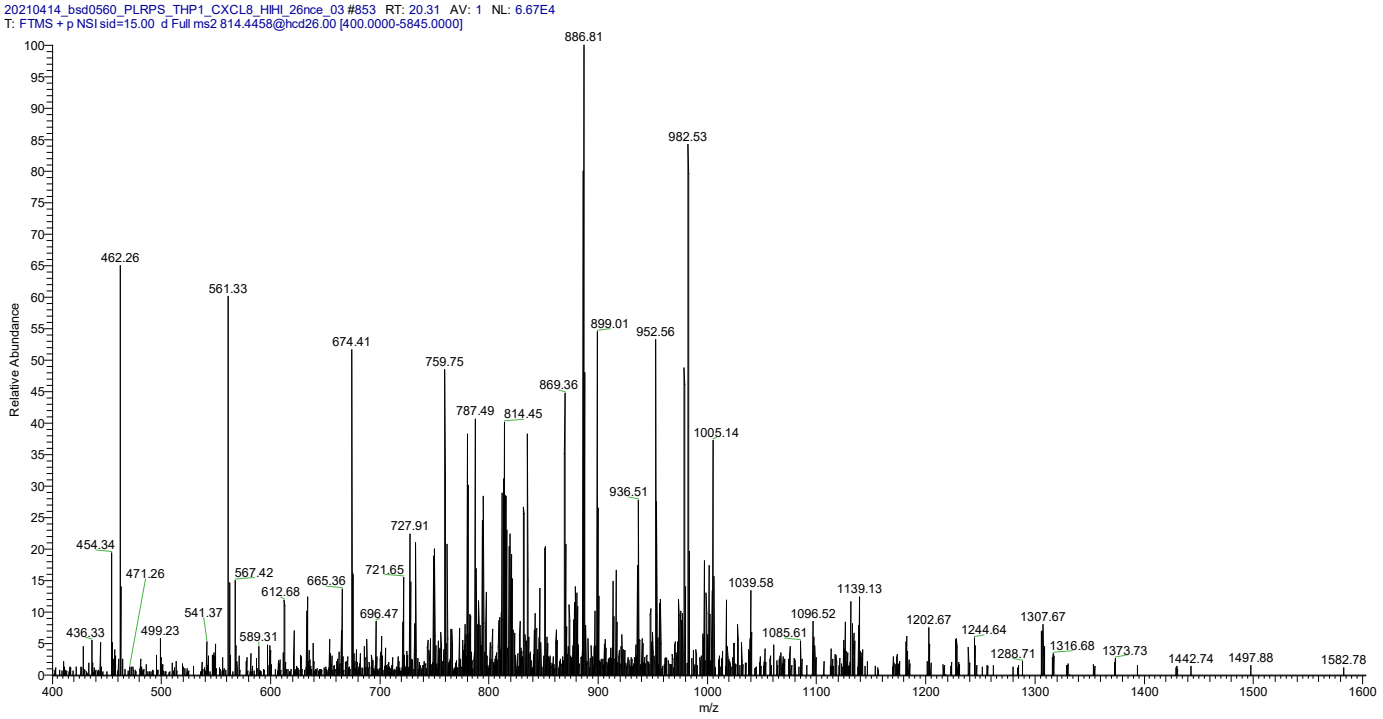
26⌊P K⌊F I K E⌊L R⌉V I E⌊S G P H⌉C⌊A⌉N⌊T⌊E⌊I⌊I⌊V⌉K⌊L 50

51⌊S⌊D⌊G⌊R E L⌉C⌋L⌉D C

Observed: 6,664.50
Theoretical: 6,664.49
Mass Diff. (Da): 0.007
Mass Diff. (ppm): 1.1
P-score: 1.1e-78

PFR00005931244:





N A V L P R S A K E L R C Q C I K T Y S K P F H P K 25

26 F I K E L R V I E S G P H C A N T E I I V K L S D C

Observed: 5,692.07
Theoretical: 5,692.06
Mass Diff. (Da): 0.008
Mass Diff. (ppm): 1.4
P-score: 7.8e-61

Protter (Omasits et al., Bioinformatics. 2013 Nov 21)