

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

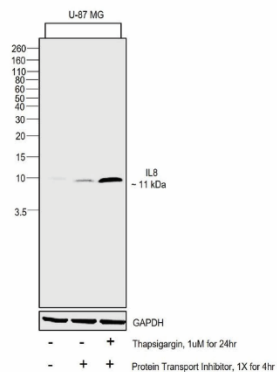
HuBMAP Platform Used: IP-MS

Protocols.io doi for Validation Protocol: 10.17504/protocols.io.btztnp6n

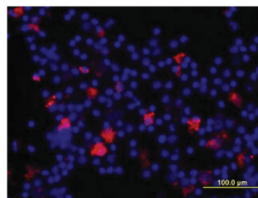
ORCID ID of submitter: 0000-0002-5631-512X, 0000-0002-6095-2797

B. Validation Data

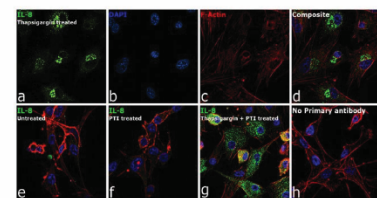
B.1. Vendor Validation: WB, IF, IHC, FC



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).



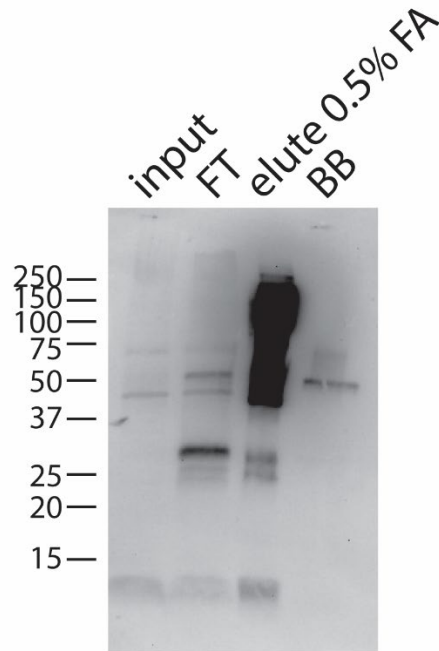
U-87 MG cells treated with 1uM of Thapsigargin for 24 hours.
Panel a: IL-8 (green)
Primary: IL-8 (CXCL8) Monoclonal Antibody (6217) (MA5-23697) at 8 µg/mL in 0.1% BSA, 4°C overnight
Secondary: Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (P# A32723), (1:2000), 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

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

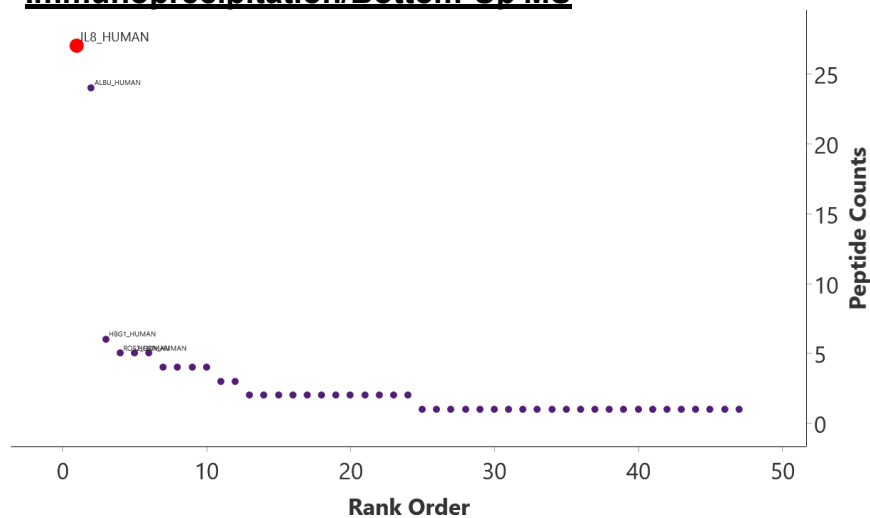
B.2. Laboratory Validation:

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: PFR000000227828, 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

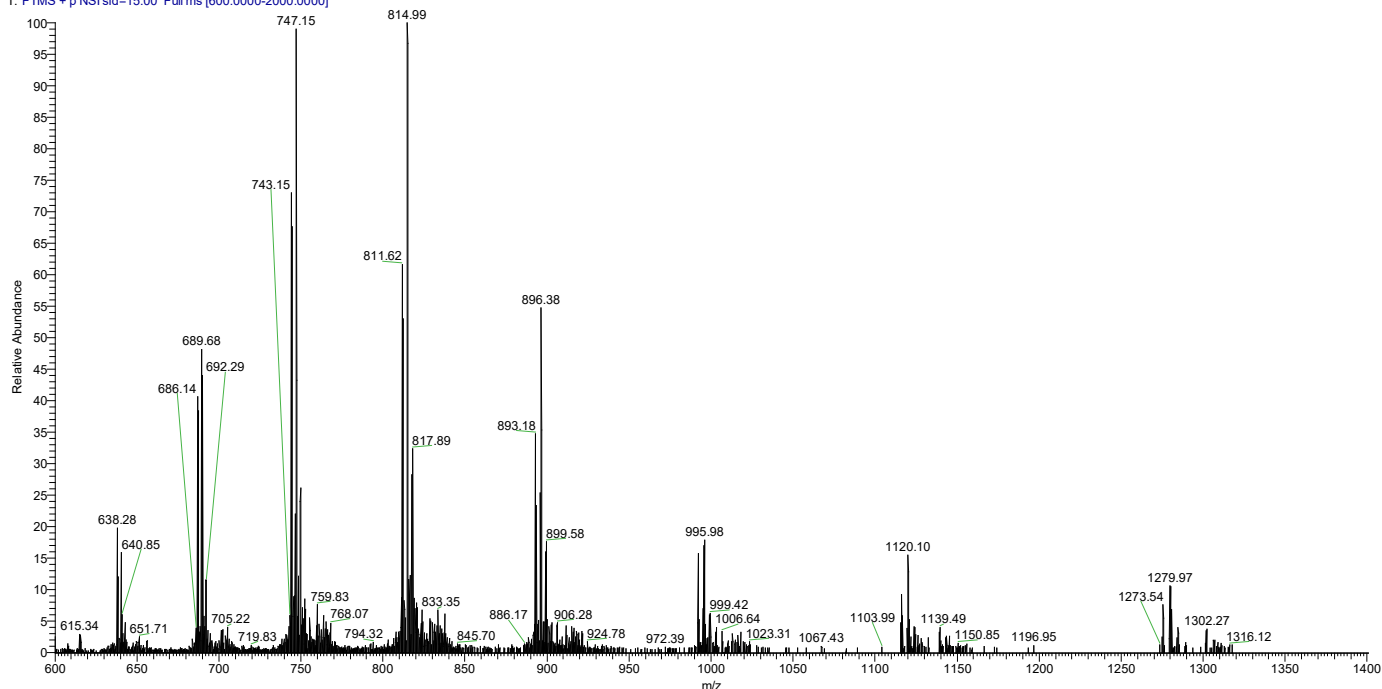
6: PFR00005931244, Interleukin-8 (1-50): 56838

AVLPRSAKELRCQCIKTYSKPFHPKFIKELRVIESGPHCANTEIIVKLSD

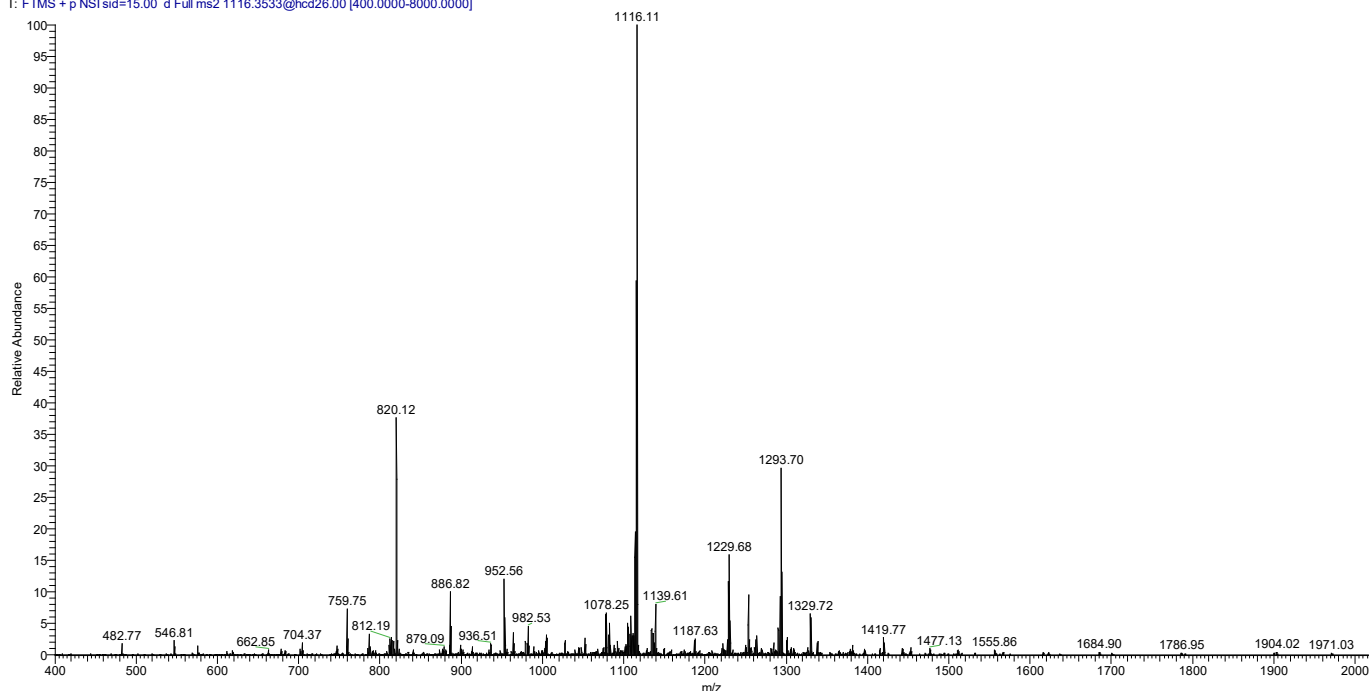
Intact Mass Profile

PFR00000072722:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #1403 RT: 24.46 AV: 1 NL: 8.10E7
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #1401 RT: 24.45 AV: 1 NL: 7.25E5
T: FTMS + p NSI sid=15.00 d Full ms2 1116.3533@hcd26.00 [400.0000-8000.0000]

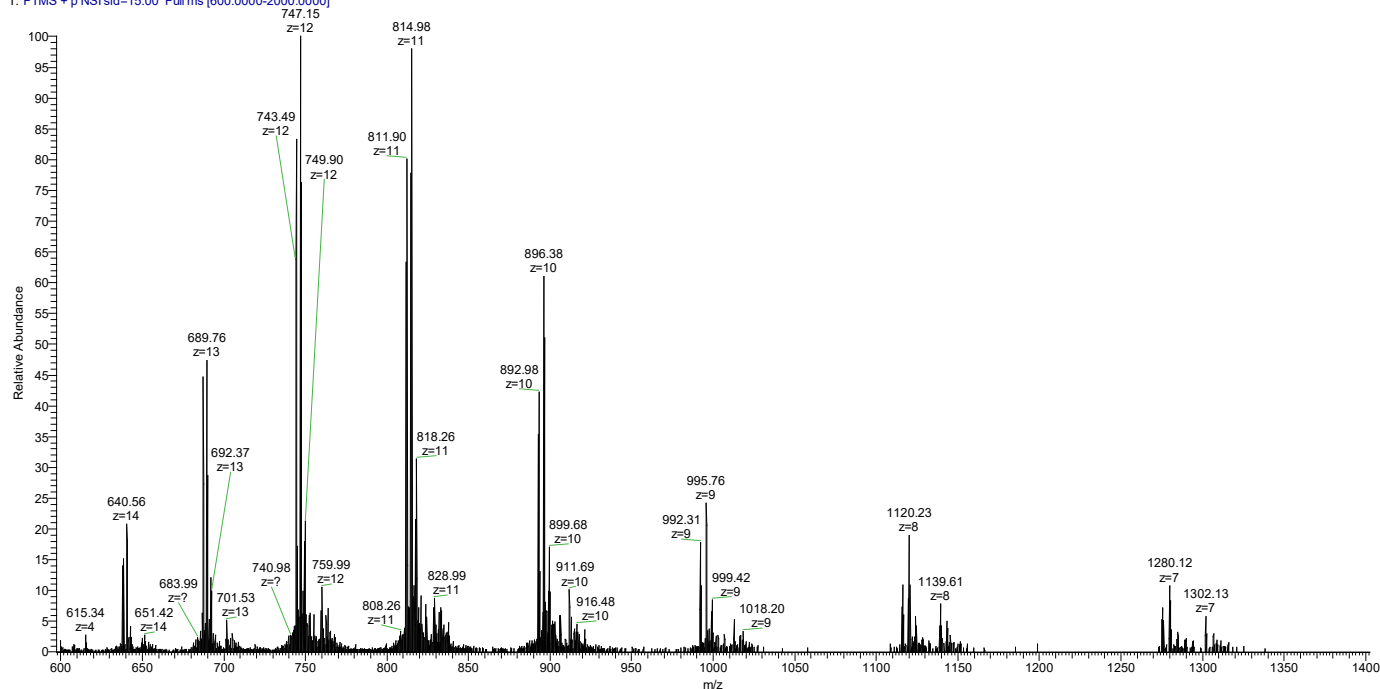


N A V L P R S A K E L R C Q C I K T Y S K P F H P K 25
26 F I K E L R V I E S G P H C A N T E I I I V K L L S D 50
51 G R E L C L D P K E N W V Q R V V E K F L L K R A E 75
76 N S C

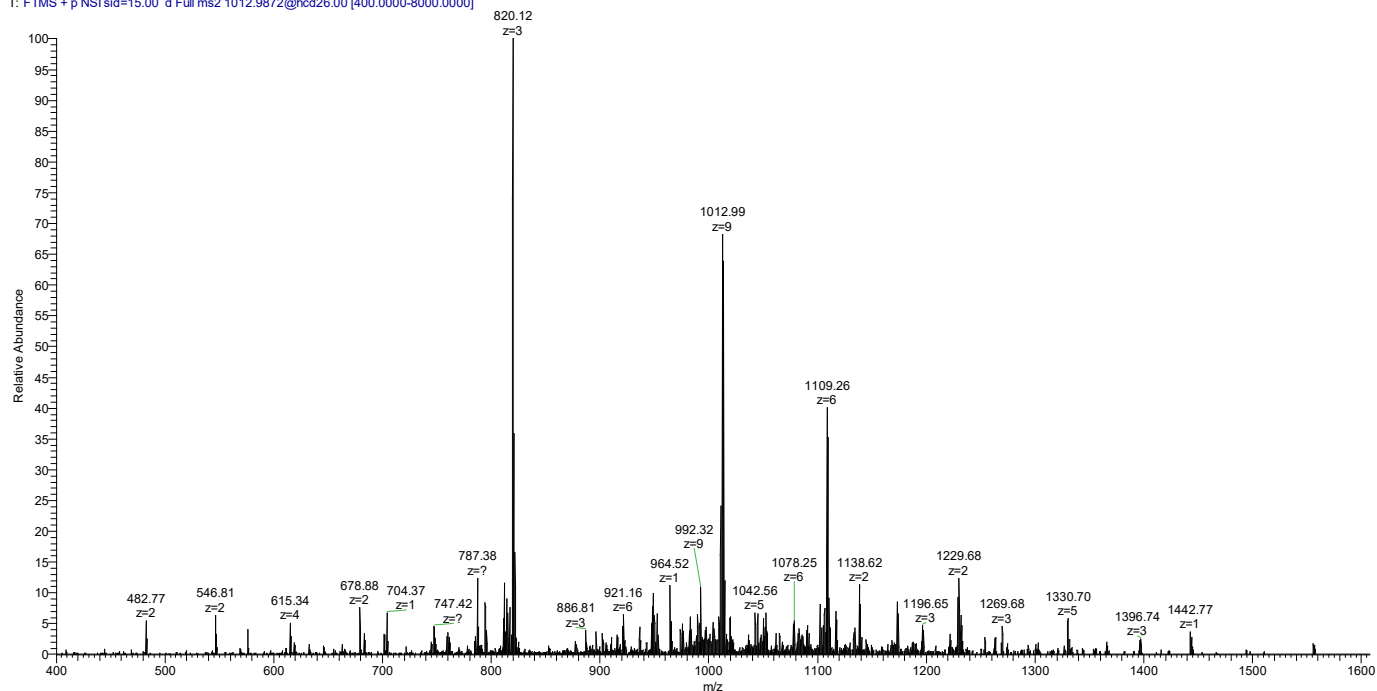
Observed: 8,916.76
Theoretical: 8,916.74
Mass Diff. (Da): 0.016
Mass Diff. (ppm): 1.83
P-score: 4.6e-89

PFR0000005811:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_01 #902 RT: 24.39 AV: 1 NL: 6.61E7
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_01 #900 RT: 24.36 AV: 1 NL: 3.14E5
T: FTMS + p NSI sid=15.00 d Full ms2 1012.9872@hcd26.00 [400.0000-8000.0000]

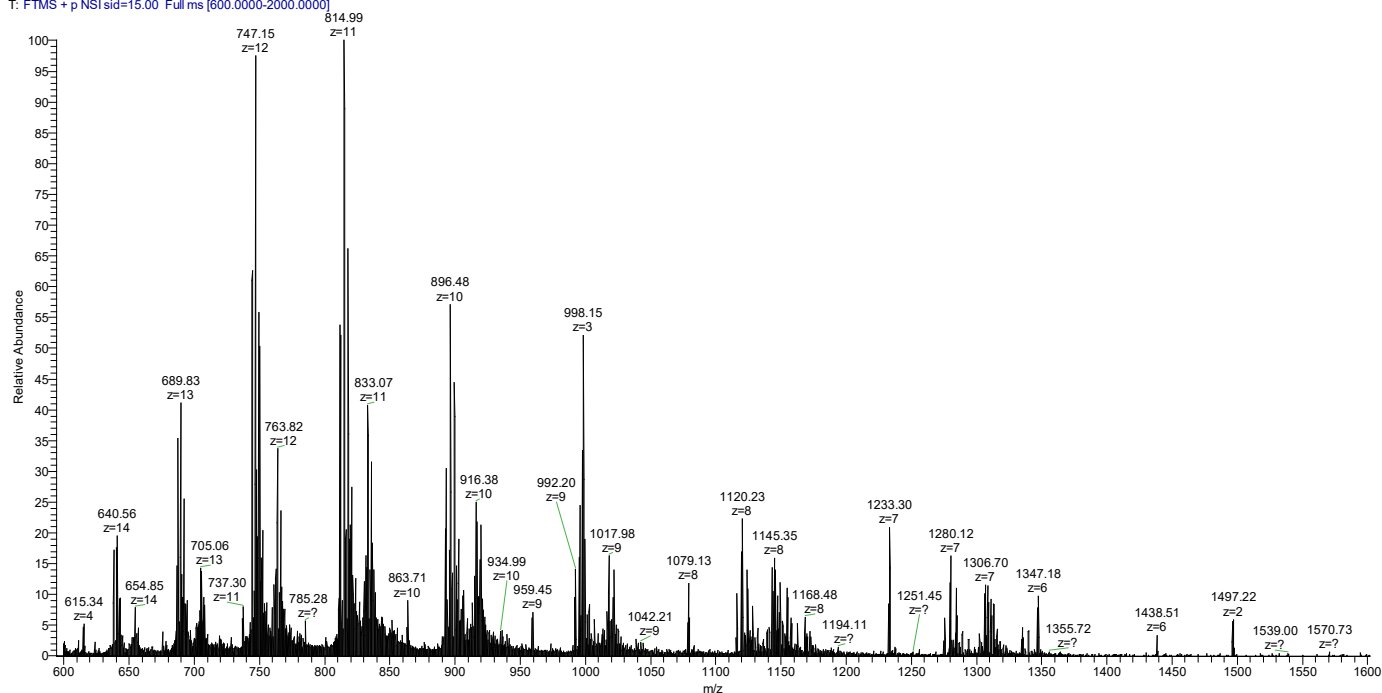


N E G A V L P R S A K E L R C Q C I I K T Y S I K P F H 25
 26 P K F I K E L R V I E S G P H C A N T E I I I V K L 50
 51 S D G R E L C L D P K E N W V Q R V V E K F L L K R 75
 76 A E N S C

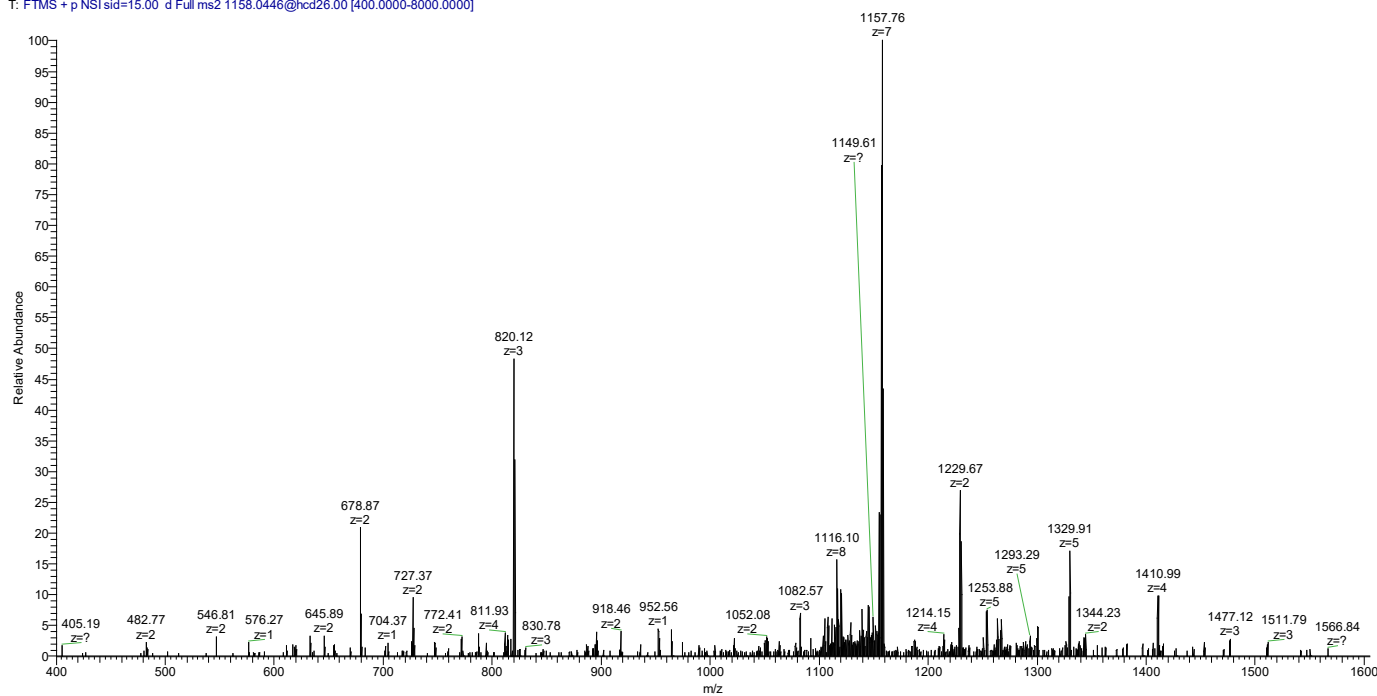
Observed: 9,102.82
 Theoretical: 9,102.81
 Mass Diff. (Da): 0.006
 Mass Diff. (ppm): 0.68
 P-score: 6.6e-70

PFR00000227828:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_02 #3043-3092 RT: 25.44-25.62 AV: 17 NL: 6.06E6
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_02 #3054 RT: 25.48 AV: 1 NL: 1.16E5
T: FTMS + p NSI sid=15.00 d Full ms2 1158.0446@hcd26.00 [400.0000-8000.0000]

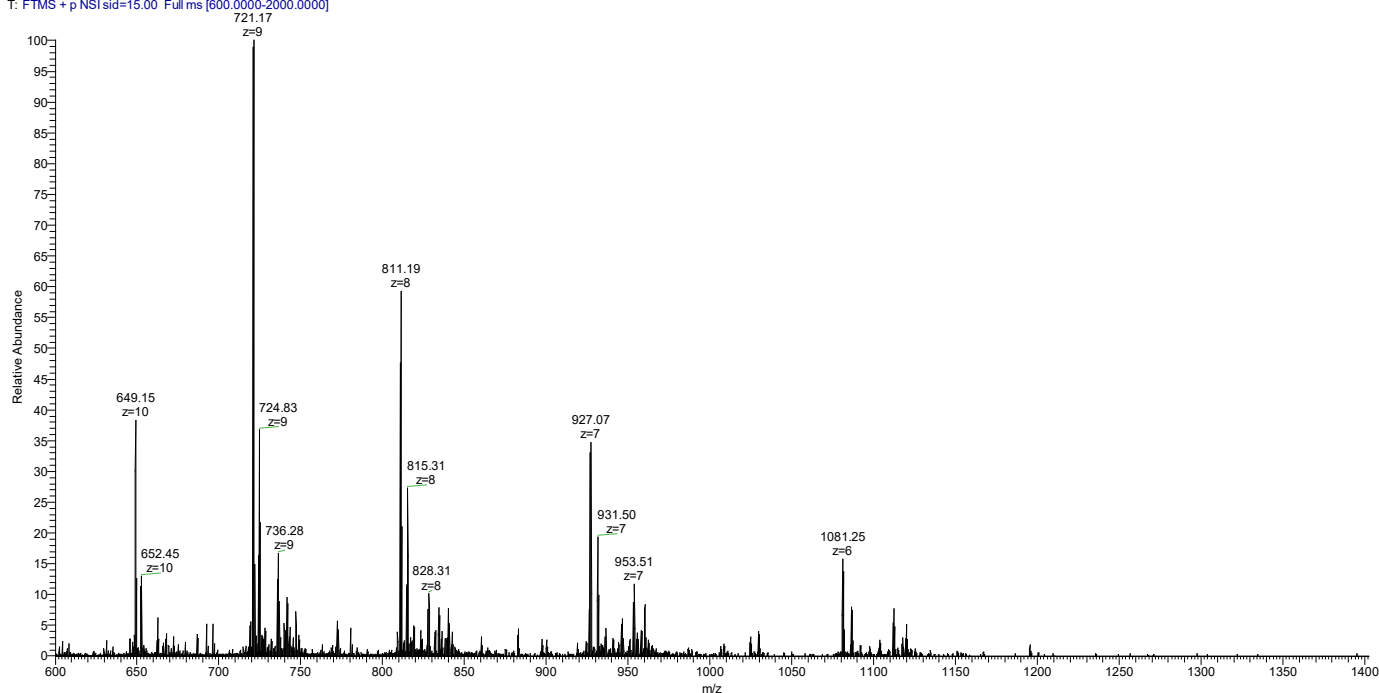


N E L R C Q C I K T Y S K P F H P K F I K E L R V I 25
26 E S G P H A N T E I I V K L S D G R E L C L D P 50
51 K E N W V Q R V V E K F L K R A E N S C

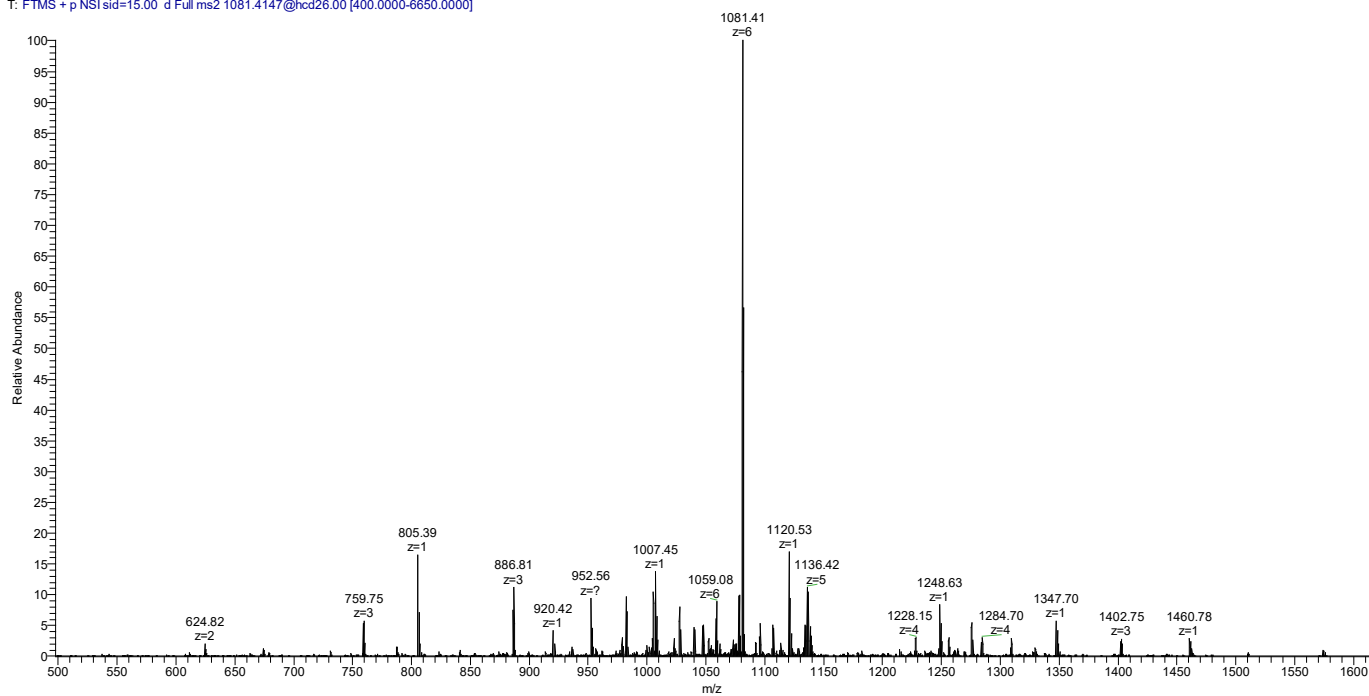
Observed: 8,094.26
Theoretical: 8,094.24
Mass Diff. (Da): 0.021
Mass Diff. (ppm): 2.6
P-score: 1.4e-31

PFR00005931242:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_01 #575 RT: 20.65 AV: 1 NL: 3.85E7
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_01 #573 RT: 20.62 AV: 1 NL: 1.09E6
T: FTMS + p NSI sid=15.00 d Full ms2 1081.4147@hcd26.00 [400.0000-6650.0000]

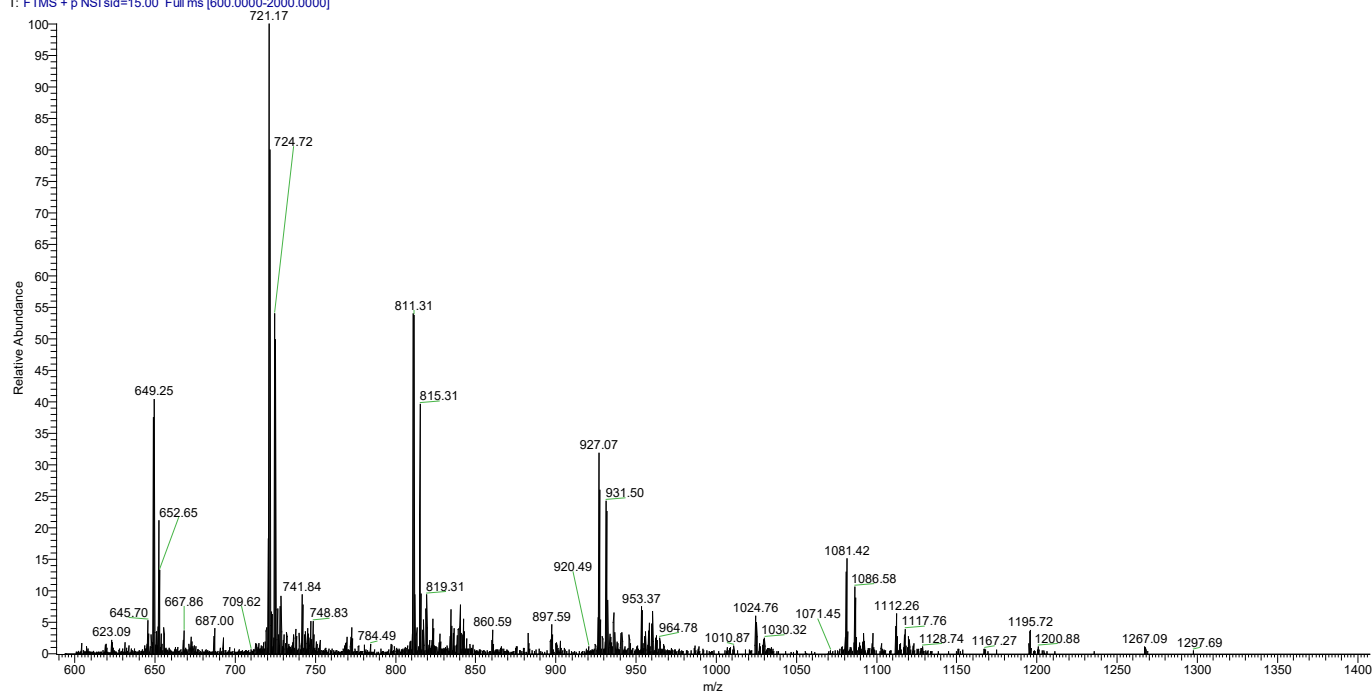


N A V L P R S A K E L R I C Q C I K T Y S K P F H P K 25
26 F I K E L R V I I E S G P H C A N T E I I I V K L L S D 50
51 G R E L C L D C

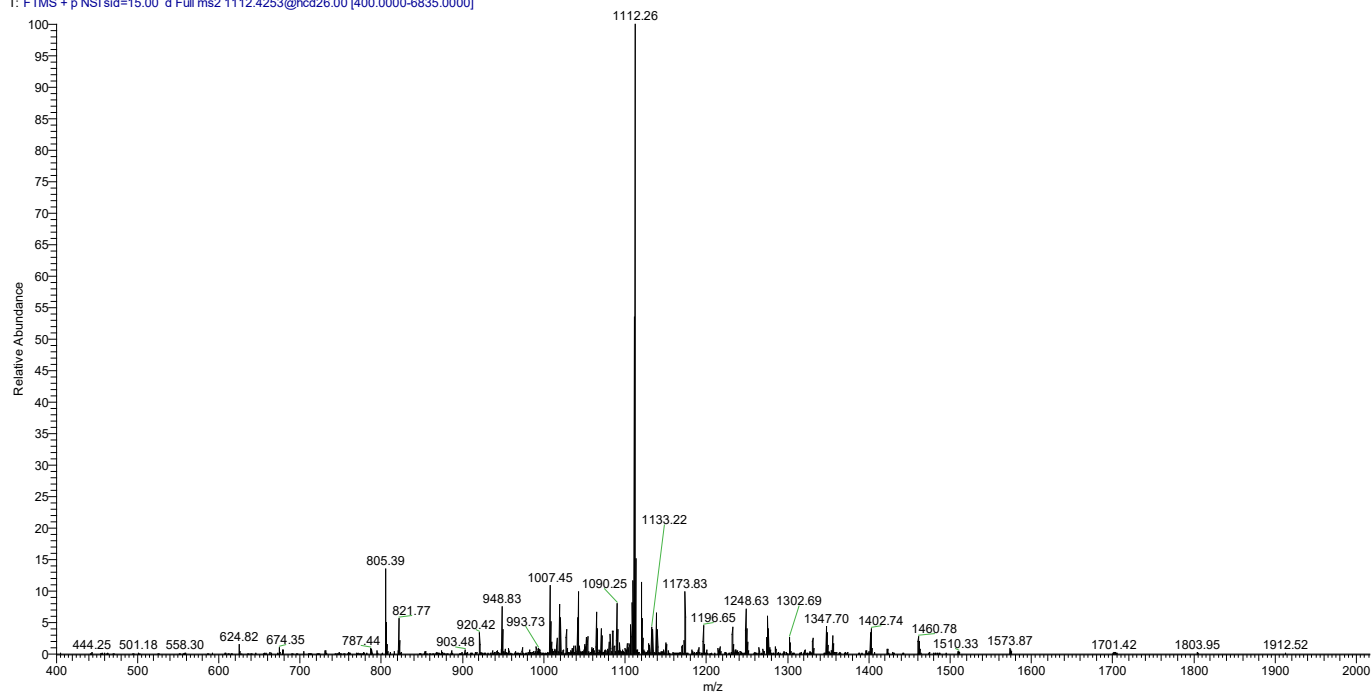
Observed: 6,478.44
Theoretical: 6,478.43
Mass Diff. (Da): 0.017
Mass Diff. (ppm): 2.6
P-score: 4.4e-97

PFR00005931243:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #921 RT: 20.83 AV: 1 NL: 3.26E7
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #920 RT: 20.82 AV: 1 NL: 4.33E5
T: FTMS + p NSI sid=15.00 d Full ms2 1112.4253@hcd26.00 [400.0000-6835.0000]

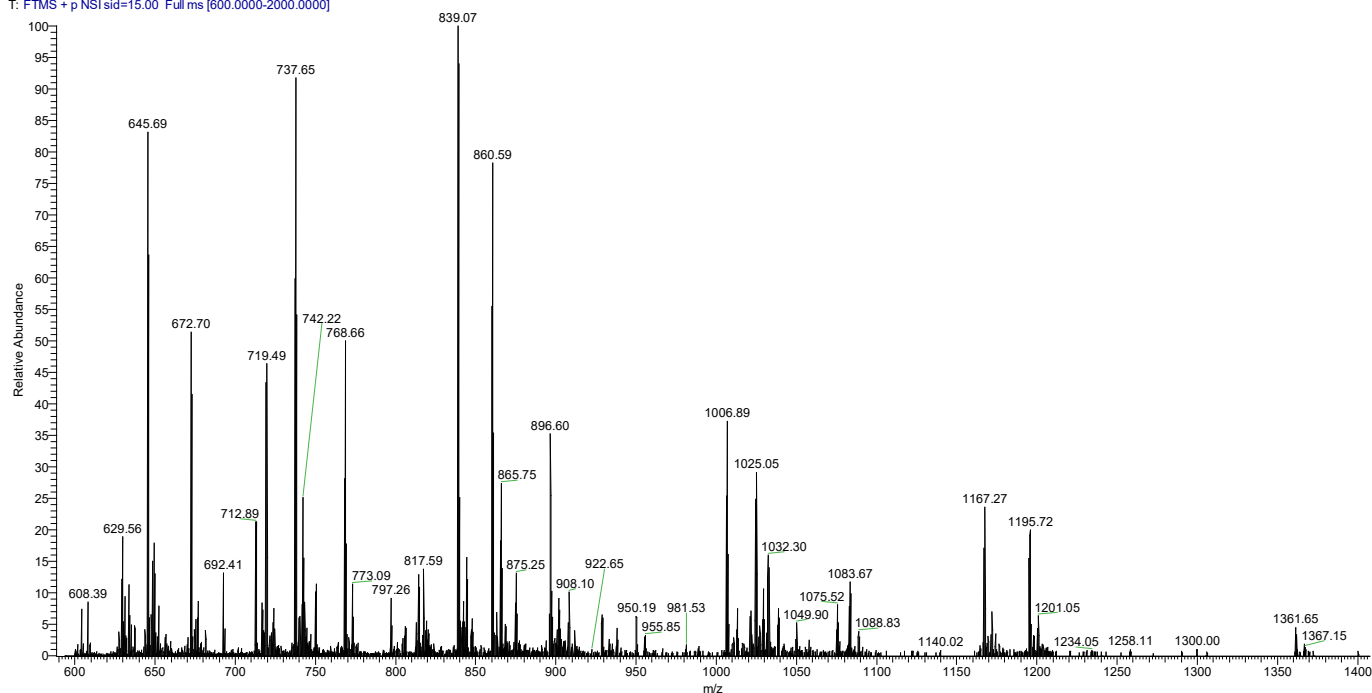


N E G **L** A V L P R S A K E **L** R **C** Q **C** I K T Y S K **L** P **F** H 25
26 **L** P K **F** I K E **L** R V I E S G P H **C** A N T E I I I V K L 50
51 **S** **D** **G** **R** E **L** **C** **L** D C

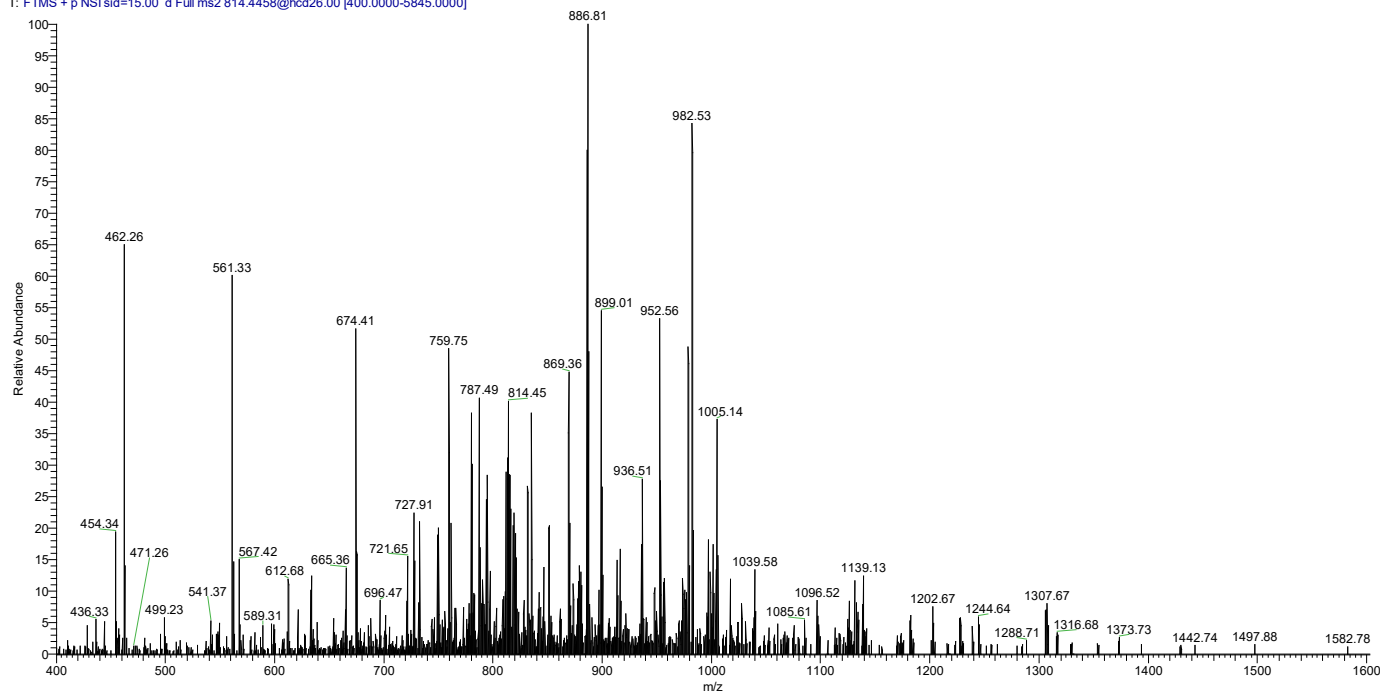
Observed: 6,664.50
Theoretical: 6,664.49
Mass Diff. (Da): 0.007
Mass Diff. (ppm): 1.1
P-score: 1.1e-78

PFR00005931244:

20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #855 RT: 20.32 AV: 1 NL: 2.89E7
T: FTMS + p NSI sid=15.00 Full ms [600.0000-2000.0000]



20210414_bsd0560_PLRPS_THP1_CXCL8_HIHI_26nce_03 #853 RT: 20.31 AV: 1 NL: 6.67E4
T: FTMS + p NSI sid=15.00 d Full ms2 814.4458@hcd26.00 [400.0000-5845.0000]



N A V L P R S A K E L R C Q I 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 A N T E I 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)

