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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Repeatability/Validation (Experiment 2) for a panel of phosphopeptide assays** | | |  |  | | **Version #: 2** | **Author: Broad Institute Proteomics Platform – Carr Lab** | | **Date: August 1, 2016** | **BRD-003** | |

# Purpose

The purpose of this document is to describe the generation of a repeatability/validation experiment for a panel of phosphopeptides.

# Scope

This procedure may be used to evaluate the repeatability/validation of IMAC enrichment and analysis by targeted mass spectrometry of phosphopeptides in a digested background matrix (cells, tissues, plasma, etc).

# Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

# Equipment

1. Microcentrifuge
2. Quantiva TSQ Mass spectrometer (Thermo)
3. Easy NanoLC 1000 liquid chromatograph (Thermo)

# Materials

1. HPLC water
2. formic acid (Fluka, 56302)
3. acetonitrile

# Reagents

**Standards:**

1. Heavy stable isotope standard mix (H-SIS): 2 pmol/uL, 30% acetonitrile/0.1% formic acid (New England Peptide)
2. Medium stable isotope standard mix (M-SIS): 1.78 pmol/uL, 30% acetonitrile/0.1% formic acid (New England Peptide)
3. Michrom mix: 50 fmol/uL digest of 6 equimolar bovine proteins (Michrom Bioresources, #PTD/00001/63) in 3% acetonitrile/5% acetic acid [Note: this product is no longer commercially available]

**Matrix:**

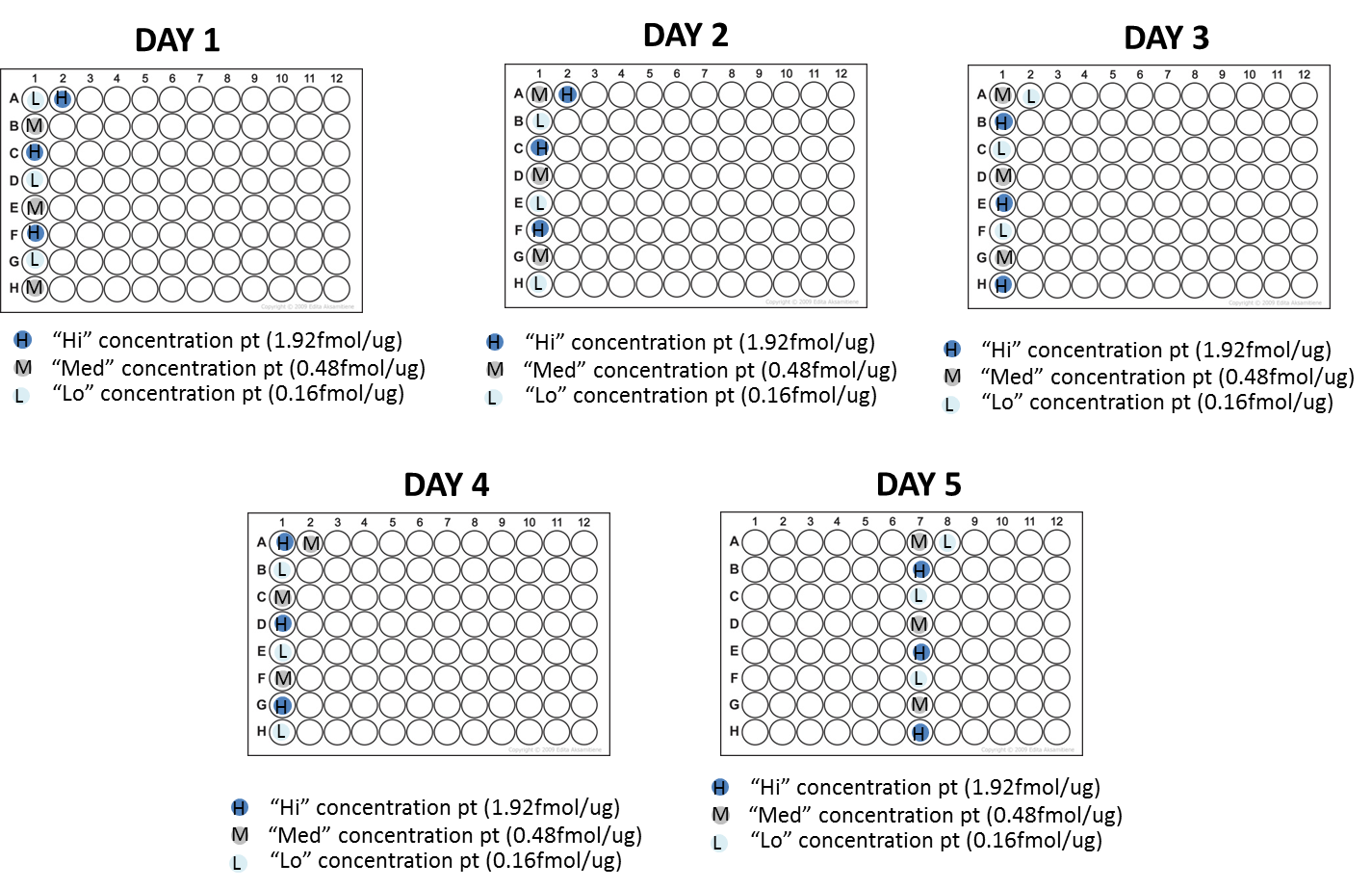
Equal mixture of two cell line lysates, A375 (melanoma – MAPK) and A549 (lung cancer – KRas), desalted and dried into wells on a 96 well plate in 500 ug aliquots as described in SOP\_Broad\_SamplePrep&PhosphoEnrich\_02, BRD-001, “**Cell Lysis, Tryptic Digestion, and Phosphopeptide Enrichment by Automated Immobilized Metal Affinity Chromatography (IMAC)”**

# Solutions

1. 50% acetonitrile
2. 80% acetonitrile
3. 3% acetonitrile/5% formic acid
4. Reconstitution buffer: 24 uL heavy peptide standard mix, 1176 uL 3% acetonitrile/5% formic acid

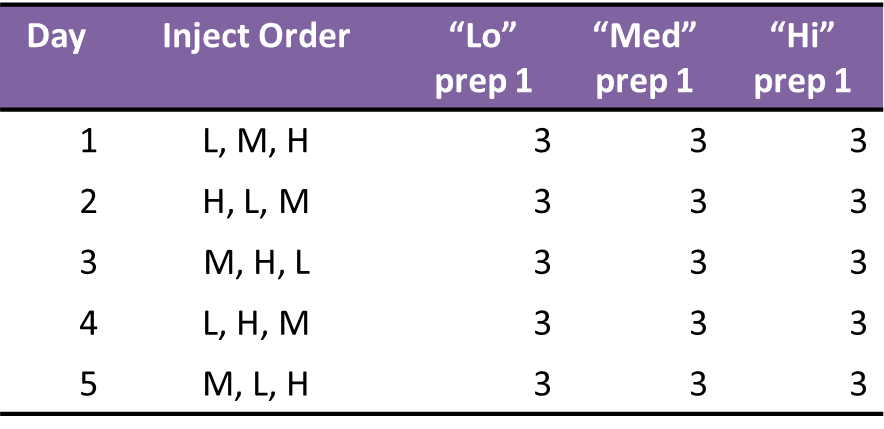
# Procedure

1. **Determination of spike levels**
   1. Following the guidelines for Experiment 2 as described in the CPTAC Assay Development Guidelines, three levels of concentration, “Lo” , “Med” and “Hi” were selected for repeatability experiments based on the reverse curve performance\.
      1. “Lo” = 0.16 fmol/ug
      2. “Med” = 0.48 fmol/ug
      3. “Hi” = 1.92 fmol/ug
   2. Five replicates of these samples were prepared by adding H-SIS phosphopeptides into digested, desalted lysate at each of the three concentration levels. These samples were then frozen. On each of 5 non-overlapping consecutive days, a set of samples were thawed and spiked with standard amount of M-SIS phosphopeptides (five replicates of each M-SIS were also prepared and frozen), enriched using automated IMAC and analyzed by targeted mass spectrometry.
2. **Preparation of Repeatability Samples - Completed before any enrichments**
   1. Cell lysates were prepared as described in SOP\_Broad\_SamplePrep&PhosphoEnrich\_02, BRD-001, “**Cell Lysis, Tryptic Digestion, and Phosphopeptide Enrichment by Automated Immobilized Metal Affinity Chromatography (IMAC)”**.
   2. Resuspend cell lysates in 50% acetonitrile to a final concentration of 2.5 mg/ml.
   3. Label 15 microcentrifuge tubes (1.5 mL): label five “Lo”, five “Med” and five “Hi”.
   4. Aliquot 660 uL of 2.5 mg/mL digested, desalted lysate to each tube.
   5. Prepare a mixture of heavy phosphospeptides from H-SIS stock mixture (2 pmol/uL) in a single tube for each of the 3 levels of concentration as described below:
      1. Lo – (0.16 fmol/ug): 2.4 uL H-SIS and 97.6 uL 80% ACN
      2. Med – (0.48 fmol/ug): 7.2 uL H-SIS and 92.8 uL 80% ACN
      3. Hi – (1.92 fmol/ug): 28.8 uL H-SIS and 71.2 uL 80% ACN
   6. Prepare five tubes for each concentration of Lo, Med, and Hi from the working stock mixtures in a single tube for each of the 3 levels of concentration as described below:
      1. Add 5 uL of the Lo (0.16 fmol/ug) to each tube labeled “Lo”.
      2. Add 5 uL of the Med (0.48 fmol/ug) to each tube labeled “Med”.
      3. Add 5 uL of the Hi (1.92 fmol/ug) to each tube labeled “Hi”.
   7. Freeze all 15 sample tubes at -80oC.
   8. Aliquot 7 uL M-SIS phosphopeptide stock (1.78 pmol/uL) into 5 tubes labeled “Med SIS” and freeze these aliquots at -80oC.
3. **Preparation of Sample Plate – Performed each day of enrichment for 5 days**
   1. Remove a single aliquot of each of the following previously prepped samples:
      1. “Med SIS” stock (7 uL @ 1.78 pmol/uL)
      2. “Hi” sample
      3. “Med” sample
      4. “Lo” sample
   2. Add 93 uL 80% ACN to “Med SIS” stock tube to bring final concentration to 120 fmol/uL.
   3. Add 5 uL of 120 fmol/uL Med SIS to each of the 3 sample tubes (“Hi”, “Med”, “Lo”).
   4. Divide each concentration point into process triplicate on the plate, as depicted below:



* 1. Follow IMAC enrichment protocol on AssayMAP Bravo as described in SOP\_Broad\_SamplePrep&PhosphoEnrich\_02, BRD-001, “**Cell Lysis, Tryptic Digestion, and Phosphopeptide Enrichment by Automated Immobilized Metal Affinity Chromatography (IMAC)”** to enrich phosphopeptides.

1. **Reconstitution of samples for analysis by mass spectrometry**
   1. After IMAC enrichment samples will be dried in autosampler vials.
   2. Resuspend dried samples in 20 uL of Reconstitution buffer (24 uL heavy peptide standard mix, 1176 uL 3% acetonitrile/5% formic acid, prepared fresh).
   3. Sonicate and vortex to resuspend phosphopeptides; centrifuge briefly (20 s at 1000 x g) to bring all liquid to the bottom of autosampler vial.
2. **NanoLC-MRM-MS analysis**
   1. set up the TSQ-Quantiva MS with Easy NanoLC1000 and the following:
      1. Q1 resolution 0.2
      2. Q3 resolution 0.7
      3. 1.5 min cycle time
      4. CID gas 1.5
      5. 0.075 x 100 mm PicoFrit Reprosil C18, 3 um, 200 Å pore size, (Dr. Maisch GmBH) PicoFrit column
      6. mobile phase A: 3% acetonitrile/0.1% formic acid, mobile phase B: 90% acetonitrile/0.1% formic acid
      7. flowrate: 300 nL/min
      8. column temperature: 50 oC
      9. gradient: 0 – 35% B in 33 min, 35 – 90%B in 3 min, hold 90%B for 6 min. (42 min total time)
      10. injection volume: 2 uL
   2. Prior to analysis, LC-MS/MS of a mixture of H-SIS peptides was performed on a high resolution MS (QExactive) to generate spectral libraries. These were imported into Skyline and used to selected MRM transitions for each peptide. The best 5-10 transition m/z’s were selected and the H-SIS was analyzed by LC-MRM-MS to determine the optimal collision energy. The best 3-5 transition ions including those required to verify the specificity of the phosphosite were then selected for further sample analysis. See Appendix 2 for scheduled MRM method used to acquire the data.
3. **Run order**
   1. Samples are analyzed in random run order as described below:



1. **Data Analysis**
   1. Raw files were imported into Skyline.
   2. Extracted Ion chromatograms (XIC) of all transition ions were integrated using a Skyline document (Skyline daily version 2.6. <https://brendanx-uw1.gs.washington.edu/labkey/project/home/software/Skyline/begin.view>).
   3. Integrated peaks were manually inspected to confirm proper integration and detection of the transitions for the corresponding light and heavy peptides.

# Referenced Documents

Protocol for automated IMAC enrichment:

[Mol Cell Proteomics.](http://www.ncbi.nlm.nih.gov/pubmed/26912667) 2016 May;15(5):1622-41. doi: 10.1074/mcp.M116.058354. Epub 2016 Feb 24. PMID:26912667

**Reduced-representation Phosphosignatures Measured by Quantitative Targeted MS Capture Cellular States and Enable Large-scale Comparison of Drug-induced Phenotypes.**

[Abelin JG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Abelin%20JG%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Patel J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Patel%20J%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Lu X](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lu%20X%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Feeney CM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Feeney%20CM%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Fagbami L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fagbami%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Creech AL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Creech%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Hu R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hu%20R%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Lam D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lam%20D%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Davison D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Davison%20D%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Pino L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pino%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Qiao JW](http://www.ncbi.nlm.nih.gov/pubmed/?term=Qiao%20JW%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Kuhn E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kuhn%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Officer A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Officer%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Li J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20J%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)2, [Abbatiello S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Abbatiello%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1,[Subramanian A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Subramanian%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Sidman R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sidman%20R%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)2, [Snyder E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Snyder%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)3, [Carr SA](http://www.ncbi.nlm.nih.gov/pubmed/?term=Carr%20SA%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)1, [Jaffe JD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Jaffe%20JD%5BAuthor%5D&cauthor=true&cauthor_uid=26912667)4

# Appendix 1. Proteins, Peptides and Pathways

|  |  |  |  |
| --- | --- | --- | --- |
| Protein | Pathway | Peptide | Phospho-site |
| ARAF | Ras mediated signaling | DSGYYWEVPPSEVQLLK | S299 |
| AURKA | Cell cycle | TTLCGTLDYLPPEMIEGR | T288 |
| AURKB | Cell cycle | KTMCGTLDYLPPEMIEGR | T232 |
| FOXO1 | Signal transduction | TSSNASTISGR | S318 |
| JUN | Immune system | NSDLLTSPDVGLLK | S63 |
| MAPK14 | Signal transduction | HTDDEMTGYVATR | T180 |
| NFKB1 | Signal transduction | DSDSVCDSGVETSFR | S932 |
| PLK1 | Cell cycle | KTLCGTPNYIAPEVLSK | T210 |
| PLK1 | Cell cycle | RSLLELHK | S137 |
| PRKCB | Signal transduction | QPVELTPTDK | T642 |
| RAF1 | Signal transduction | ASDDGKLTDPSK | S43 |
| RAF1 | Signal transduction | SASEPSLHR | S621 |
| RB1 | Cell cycle | TAVIPINGSPR | S249 |
| RICTOR | Signal transduction | AQSLKAPSIATIK | S1302 |
| RPS6 | Disease | LSSLRASTSK | S235 |
| RPS6 | Disease | LSSLRASTSK | S236 |
| RPS6 | Disease | LSSLRASTSK | S240 |
| STAT3 | Signal transduction | FICVTPTTCSNTIDLPMSPR | S727 |
| SUFU | Development pathways | DSLESDSSTAIIPHELIR | S346 |
| TP53BP1 | DNA repair | QYTESQLR | S1778 |

# Appendix 2. Scheduled MRM Method

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound | Start Time (min) | End Time (min) | Polarity | Precursor (m/z) | Product (m/z) | Collision Energy (V) |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 469.9251 | 432.2817 | 17.9 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 469.9251 | 632.3978 | 15.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 469.9251 | 679.3175 | 15.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 469.9251 | 729.4505 | 20.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 471.9318 | 638.4179 | 15.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 471.9318 | 735.4706 | 20.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 472.5965 | 440.2959 | 17.9 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 472.5965 | 640.412 | 15.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 472.5965 | 679.3175 | 15.4 |
| AQS[+80.0]LKAPSIATIK | 20.5 | 25 | Positive | 472.5965 | 737.4647 | 20.4 |
| AQS[+80.0]LKAPSIATIK2 | 20.5 | 25 | Positive | 471.9318 | 438.3018 | 17.9 |
| AQS[+80.0]LKAPSIATIK2 | 20.5 | 25 | Positive | 471.9318 | 679.3175 | 15.4 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 657.2847 | 371.1197 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 657.2847 | 538.2669 | 22.1 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 657.2847 | 547.2722 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 657.2847 | 660.3563 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 660.2947 | 553.2923 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 660.2947 | 666.3764 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 661.2918 | 371.1197 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 661.2918 | 542.274 | 22.1 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 661.2918 | 555.2864 | 29.6 |
| AS[+80.0]DDGKLTDPSK | 12.5 | 16 | Positive | 661.2918 | 668.3705 | 29.6 |
| AS[+80.0]DDGKLTDPSK2 | 12.5 | 16 | Positive | 660.2947 | 371.1197 | 29.6 |
| AS[+80.0]DDGKLTDPSK2 | 12.5 | 16 | Positive | 660.2947 | 541.277 | 22.1 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1045.48 | 982.3941 | 27.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1045.48 | 1010.588 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1045.48 | 1080.371 | 27.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1045.48 | 1109.656 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1048.49 | 1016.608 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1048.49 | 1115.677 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1049.487 | 982.3941 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1049.487 | 1018.602 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1049.487 | 1080.371 | 27.8 |
| DS[+80.0]GYYWEVPPSEVQLLK | 31 | 35.5 | Positive | 1049.487 | 1117.671 | 32.8 |
| DS[+80.0]GYYWEVPPSEVQLLK2 | 31 | 35.5 | Positive | 1048.49 | 982.3941 | 27.8 |
| DS[+80.0]GYYWEVPPSEVQLLK2 | 31 | 35.5 | Positive | 1048.49 | 1080.371 | 27.8 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 688.3279 | 427.1823 | 21.8 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 688.3279 | 764.4413 | 26.8 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 688.3279 | 877.5254 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 688.3279 | 990.6095 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 690.3346 | 883.5455 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 690.3346 | 996.6296 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 691.664 | 427.1823 | 21.8 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 691.664 | 774.4496 | 26.8 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 691.664 | 887.5337 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR | 26 | 30 | Positive | 691.664 | 1000.618 | 29.3 |
| DS[+80.0]LESDSSTAIIPHELIR2 | 26 | 30 | Positive | 690.3346 | 427.1823 | 21.8 |
| DS[+80.0]LESDSSTAIIPHELIR2 | 26 | 30 | Positive | 690.3346 | 770.4615 | 26.8 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 870.8246 | 875.3659 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 870.8246 | 962.3979 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 870.8246 | 1077.425 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 870.8246 | 1139.479 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 870.8246 | 1237.455 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 875.8287 | 885.3741 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 875.8287 | 972.4062 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 875.8287 | 1087.433 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 875.8287 | 1149.487 | 36.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR | 19 | 23 | Positive | 875.8287 | 1247.464 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR2 | 19 | 23 | Positive | 873.8346 | 881.386 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR2 | 19 | 23 | Positive | 873.8346 | 968.418 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR2 | 19 | 23 | Positive | 873.8346 | 1083.445 | 26.9 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR2 | 19 | 23 | Positive | 873.8346 | 1145.499 | 29.4 |
| DSDSVC[+57.0]DSGVETS[+80.0]FR2 | 19 | 23 | Positive | 873.8346 | 1243.476 | 29.4 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 525.8764 | 446.2722 | 14.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 525.8764 | 598.2103 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 525.8764 | 609.3355 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 525.8764 | 749.3941 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 525.8764 | 869.3094 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 529.2124 | 456.2804 | 14.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 529.2124 | 598.2103 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 529.2124 | 619.3438 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 529.2124 | 759.4023 | 19.5 |
| HTDDEMT[+80.0]GYVATR | 17 | 21 | Positive | 529.2124 | 869.3094 | 19.5 |
| HTDDEMT[+80.0]GYVATR2 | 17 | 21 | Positive | 527.8831 | 452.2923 | 14.5 |
| HTDDEMT[+80.0]GYVATR2 | 17 | 21 | Positive | 527.8831 | 598.2103 | 19.5 |
| HTDDEMT[+80.0]GYVATR2 | 17 | 21 | Positive | 527.8831 | 615.3556 | 19.5 |
| HTDDEMT[+80.0]GYVATR2 | 17 | 21 | Positive | 527.8831 | 755.4142 | 19.5 |
| HTDDEMT[+80.0]GYVATR2 | 17 | 21 | Positive | 527.8831 | 869.3094 | 19.5 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 657.6601 | 672.3927 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 657.6601 | 741.3001 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 657.6601 | 743.4298 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 657.6601 | 952.3958 | 23.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 659.6668 | 678.4128 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 659.6668 | 749.4499 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 660.3315 | 680.4069 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 660.3315 | 741.3001 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 660.3315 | 751.444 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK | 23.5 | 28 | Positive | 660.3315 | 952.3958 | 23.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK2 | 23.5 | 28 | Positive | 659.6668 | 741.3001 | 18.4 |
| KT[+80.0]LC[+57.0]GTPNYIAPEVLSK2 | 23.5 | 28 | Positive | 659.6668 | 952.3958 | 23.4 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 730.9926 | 416.2051 | 30.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 730.9926 | 464.7315 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 730.9926 | 831.4029 | 28.1 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 730.9926 | 928.4557 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 730.9926 | 987.3675 | 28.1 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 734.3287 | 421.2092 | 30.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 734.3287 | 469.7356 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 734.3287 | 841.4112 | 28.1 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 734.3287 | 938.4639 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR | 28 | 32 | Positive | 734.3287 | 987.3675 | 28.1 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR2 | 28 | 32 | Positive | 732.9993 | 419.2152 | 30.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR2 | 28 | 32 | Positive | 732.9993 | 467.7415 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR2 | 28 | 32 | Positive | 732.9993 | 837.423 | 28.1 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR2 | 28 | 32 | Positive | 732.9993 | 934.4758 | 20.6 |
| KT[+80.0]MC[+57.0]GTLDYLPPEMIEGR2 | 28 | 32 | Positive | 732.9993 | 987.3675 | 28.1 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 565.2843 | 422.2245 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 565.2843 | 637.3069 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 565.2843 | 762.4468 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 565.2843 | 849.4789 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 565.2843 | 885.4789 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 569.2914 | 430.2387 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 569.2914 | 637.3069 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 569.2914 | 770.461 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 569.2914 | 857.4931 | 31.5 |
| LS[+80.0]SLRASTSK | 12 | 17 | Positive | 569.2914 | 885.4789 | 31.5 |
| LS[+80.0]SLRASTSK2 | 12 | 17 | Positive | 568.2943 | 428.2447 | 31.5 |
| LS[+80.0]SLRASTSK2 | 12 | 17 | Positive | 568.2943 | 637.3069 | 31.5 |
| LS[+80.0]SLRASTSK2 | 12 | 17 | Positive | 568.2943 | 768.467 | 31.5 |
| LS[+80.0]SLRASTSK2 | 12 | 17 | Positive | 568.2943 | 855.499 | 31.5 |
| LS[+80.0]SLRASTSK2 | 12 | 17 | Positive | 568.2943 | 885.4789 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 565.2843 | 422.2245 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 565.2843 | 637.3069 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 565.2843 | 762.4468 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 565.2843 | 885.4789 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 565.2843 | 929.4452 | 19 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 569.2914 | 430.2387 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 569.2914 | 637.3069 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 569.2914 | 770.461 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 569.2914 | 885.4789 | 31.5 |
| LSS[+80.0]LRASTSK | 12 | 17 | Positive | 569.2914 | 937.4594 | 19 |
| LSS[+80.0]LRASTSK2 | 12 | 17 | Positive | 568.2943 | 428.2447 | 31.5 |
| LSS[+80.0]LRASTSK2 | 12 | 17 | Positive | 568.2943 | 637.3069 | 31.5 |
| LSS[+80.0]LRASTSK2 | 12 | 17 | Positive | 568.2943 | 768.467 | 31.5 |
| LSS[+80.0]LRASTSK2 | 12 | 17 | Positive | 568.2943 | 885.4789 | 31.5 |
| LSS[+80.0]LRASTSK2 | 12 | 17 | Positive | 568.2943 | 935.4653 | 19 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 565.2843 | 502.1909 | 19 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 565.2843 | 557.3406 | 26.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 565.2843 | 842.4132 | 21.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 565.2843 | 885.4789 | 31.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 565.2843 | 929.4452 | 19 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 569.2914 | 510.2051 | 19 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 569.2914 | 557.3406 | 26.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 569.2914 | 850.4274 | 21.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 569.2914 | 885.4789 | 31.5 |
| LSSLRAS[+80.0]TSK | 12 | 17 | Positive | 569.2914 | 937.4594 | 19 |
| LSSLRAS[+80.0]TSK2 | 12 | 17 | Positive | 568.2943 | 508.211 | 19 |
| LSSLRAS[+80.0]TSK2 | 12 | 17 | Positive | 568.2943 | 557.3406 | 26.5 |
| LSSLRAS[+80.0]TSK2 | 12 | 17 | Positive | 568.2943 | 848.4333 | 21.5 |
| LSSLRAS[+80.0]TSK2 | 12 | 17 | Positive | 568.2943 | 885.4789 | 31.5 |
| LSSLRAS[+80.0]TSK2 | 12 | 17 | Positive | 568.2943 | 935.4653 | 19 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 776.3869 | 741.4505 | 28.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 776.3869 | 908.4489 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 776.3869 | 1009.497 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 776.3869 | 1122.581 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 779.397 | 1015.517 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 779.397 | 1128.601 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 780.394 | 749.4647 | 28.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 780.394 | 916.4631 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 780.394 | 1017.511 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK | 28 | 32 | Positive | 780.394 | 1130.595 | 23.7 |
| NSDLLTS[+80.0]PDVGLLK2 | 28 | 32 | Positive | 779.397 | 747.4706 | 28.7 |
| NSDLLTS[+80.0]PDVGLLK2 | 28 | 32 | Positive | 779.397 | 914.469 | 23.7 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 604.2839 | 460.2402 | 25.3 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 604.2839 | 543.2773 | 17.8 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 604.2839 | 641.2542 | 17.8 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 604.2839 | 754.3383 | 20.3 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 607.294 | 647.2743 | 17.8 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 607.294 | 760.3584 | 20.3 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 608.291 | 468.2544 | 25.3 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 608.291 | 551.2915 | 25.3 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 608.291 | 649.2684 | 17.8 |
| QPVELT[+80.0]PTDK | 16.5 | 23 | Positive | 608.291 | 762.3525 | 20.3 |
| QPVELT[+80.0]PTDK2 | 16.5 | 23 | Positive | 607.294 | 466.2603 | 25.3 |
| QPVELT[+80.0]PTDK2 | 16.5 | 23 | Positive | 607.294 | 549.2974 | 17.8 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 359.1935 | 339.2139 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 359.1935 | 452.298 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 359.1935 | 526.2984 | 14.6 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.2002 | 339.2139 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.2002 | 452.298 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.2002 | 532.3185 | 14.6 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.8649 | 339.2139 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.8649 | 452.298 | 17.1 |
| RS[+80.0]LLELHK | 17 | 21 | Positive | 361.8649 | 534.3126 | 14.6 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 532.232 | 357.1405 | 20.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 532.232 | 609.3467 | 25.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 532.232 | 738.3893 | 22.9 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 535.2421 | 357.1405 | 20.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 535.2421 | 615.3668 | 25.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 535.2421 | 744.4094 | 22.9 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 537.2362 | 357.1405 | 20.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 537.2362 | 619.355 | 25.4 |
| SAS[+80.0]EPSLHR | 12 | 16.5 | Positive | 537.2362 | 748.3976 | 22.9 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 602.8079 | 410.6893 | 20.3 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 602.8079 | 722.3944 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 602.8079 | 820.3713 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 602.8079 | 933.4554 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 605.818 | 826.3914 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 605.818 | 939.4755 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 607.8121 | 415.6934 | 20.3 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 607.8121 | 732.4027 | 27.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 607.8121 | 830.3796 | 17.8 |
| TAVIPINGS[+80.0]PR | 19 | 23 | Positive | 607.8121 | 943.4636 | 17.8 |
| TAVIPINGS[+80.0]PR2 | 19 | 23 | Positive | 605.818 | 413.6994 | 20.3 |
| TAVIPINGS[+80.0]PR2 | 19 | 23 | Positive | 605.818 | 728.4145 | 17.8 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 580.7508 | 533.3042 | 27 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 580.7508 | 541.1654 | 19.5 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 580.7508 | 620.3362 | 24.5 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 580.7508 | 691.3733 | 27 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 583.7609 | 626.3563 | 24.5 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 583.7609 | 697.3935 | 27 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 585.7549 | 541.1654 | 19.5 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 585.7549 | 543.3125 | 27 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 585.7549 | 630.3445 | 24.5 |
| TSS[+80.0]NASTISGR | 11 | 15 | Positive | 585.7549 | 701.3816 | 27 |
| TSS[+80.0]NASTISGR2 | 11 | 15 | Positive | 583.7609 | 539.3243 | 27 |
| TSS[+80.0]NASTISGR2 | 11 | 15 | Positive | 583.7609 | 541.1654 | 19.5 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 715.9914 | 416.2051 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 715.9914 | 464.7315 | 20.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 715.9914 | 616.2759 | 22.6 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 715.9914 | 831.4029 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 715.9914 | 928.4557 | 20.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 719.3275 | 421.2092 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 719.3275 | 469.7356 | 20.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 719.3275 | 616.2759 | 22.6 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 719.3275 | 841.4112 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR | 31 | 35.5 | Positive | 719.3275 | 938.4639 | 20.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR2 | 31 | 35.5 | Positive | 717.9981 | 419.2152 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR2 | 31 | 35.5 | Positive | 717.9981 | 467.7415 | 20.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR2 | 31 | 35.5 | Positive | 717.9981 | 616.2759 | 22.6 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR2 | 31 | 35.5 | Positive | 717.9981 | 837.423 | 30.1 |
| TT[+80.0]LC[+57.0]GTLDYLPPEMIEGR2 | 31 | 35.5 | Positive | 717.9981 | 934.4758 | 20.1 |