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| STANDARD OPERATING PROCEDURE |
| |  |  | | --- | --- | | **Title: Reverse Response Curve Preparation and Analysis (Experiment 1) for a panel of Phosphopeptide Assays** | | |  |  | | **Version #: 1** | **Author: Broad Inst Proteomics Platform** | | **Date: June 14, 2016** | **BRD-002** | |

# Purpose

The purpose of this document is to describe the preparation of a reverse response curve for a panel of phosphopeptides.

# Scope

This procedure may be used to prepare a reverse curve of phosphopeptides in a digested background matrix (cells, tissues, plasma, etc) and analyze the mixture after IMAC enrichment by targeted mass spectrometry.

# 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 grade 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)

1. Medium stable isotope standard mix (M-SIS): 1.78 pmol/uL, 30% acetonitrile/0.1% formic acid

(New England Peptide)

1. 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\_01, 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. **Preparation of Reverse Curve (refer to Table 1)**
   1. Make standard solution (20 fmol/uL medium stable isotope standard mix (M-SIS) in 80% acetonitrile/0.1% formic acid). Add 14 uL of M-SIS to 1236 uL 80% acetonitrile/0.1% formic acid.
   2. Add 112.5 uL standard solution to 8 microcentrifuge tubes (1.5 mL). Cap one tube (tube 1) and set aside to use as blank.
   3. Add 37.5 uL H-SIS to tube 8, final concentration 500 fmol/uL.
   4. Add 0.4 uL M-SIS to tube 8 to restore medium SIS concentration to 20 fmol/uL after the addition of H-SIS.
   5. Transfer 37.5 uL from tube 8 to tube 7 for final heavy SIS concentration of 125 fmol/uL in tube 7. Continue serial dilution, removing 37.5 uL from previous tube and adding to successive tube for a total of 7 tubes.

Table 1. Concentrations of M-SIS and H-SIS in reverse curve

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **pt** | **total M-SIS (fmol)** | **total H-SIS (fmol)** | **M-SIS conc (fmol/ug)** | **H-SIS conc (fmol/ug)** |
| **1** | 200 | 0 | 0.4 | 0 |
| **2** | 200 | 1.2 | 0.4 | 0.002 |
| **3** | 200 | 5 | 0.4 | 0.01 |
| **4** | 200 | 20 | 0.4 | 0.04 |
| **5** | 200 | 78 | 0.4 | 0.16 |
| **6** | 200 | 312 | 0.4 | 0.63 |
| **7** | 200 | 1250 | 0.4 | 2.5 |
| **8** | 200 | 5000 | 0.4 | 10 |

1. **Preparation of Sample Plate (refer to Table 2)**
   1. Add 88 uL 50% acetonitrile to the sample plate containing dried digested desalted cell lysate prepared using SOP\_Broad\_SamplePrep&PhosphoEnrich\_01, BRD-001, “**Cell Lysis, Tryptic Digestion, and Phosphopeptide Enrichment by Automated Immobilized Metal Affinity Chromatography (IMAC)”**. Refer to the plate map in Table 2.
   2. Seal plate well with aluminum foil seal, sonicate and vortex to resuspend peptides. Centrifuge for 30 s at 1500 x g to bring liquid into wells.
   3. Remove seal, add 132 uL 100% acetonitrile to make the solution 80% acetonitrile.
   4. Seal plate well with a fresh aluminum foil seal, sonicate and vortex to resuspend peptides. Centrifuge for 30 s at 1500 x g to bring liquid into wells. Each well now contains 500 ug cell lysate peptides resuspended in 220 uL 80% acetonitrile.
   5. Remove seal, add 10 uL from each curve tube prepared in 1. above and pipet up and down five times to mix.

***For example, dispense 10 uL of curve point 1 (0 fmol/uL H-SIS, 20 fmol/uL M-SIS) into wells A1 A2 A3, 10 uL each of curve point 2 (0.02 fmol/uL H-SIS, 20 fmol/uL M-SIS) into wells B1 B2 B3 and continue in a similar manner for each curve point.***

* 1. Follow SOP\_Broad\_SamplePrep&PhosphoEnrich\_01, BRD-001, “**Cell Lysis, Tryptic Digestion, and Phosphopeptide Enrichment by Automated Immobilized Metal Affinity Chromatography (IMAC)”** to enrich phosphopeptides.

Table 2. Curve platemap and concentrations

|  |  |  |  |
| --- | --- | --- | --- |
|  | **curve** | **concentration** | **(fmol/ug)** |
|  |  |  |  |
|  | **1** | **2** | **3** |
| **A** | 0 | 0 | 0 |
| **B** | 0.002 | 0.002 | 0.002 |
| **C** | 0.01 | 0.01 | 0.01 |
| **D** | 0.04 | 0.04 | 0.04 |
| **E** | 0.16 | 0.16 | 0.16 |
| **F** | 0.63 | 0.63 | 0.63 |
| **G** | 2.5 | 2.5 | 2.5 |
| **H** | 10 | 10 | 10 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **plate** | **map** |  |
|  |  |  |  |
|  | **1** | **2** | **3** |
| **A** | pt 1-1 | pt 1-2 | pt 1-3 |
| **B** | pt 2-1 | pt 2-2 | pt 2-3 |
| **C** | pt 3-1 | pt 3-2 | pt 3-3 |
| **D** | pt 4-1 | pt 4-2 | pt 4-3 |
| **E** | pt 5-1 | pt 5-2 | pt 5-3 |
| **F** | pt 6-1 | pt 6-2 | pt 6-3 |
| **G** | pt 7-1 | pt 7-2 | pt 7-3 |
| **H** | pt 8-1 | pt 8-2 | pt 8-3 |

1. **Reconstitution of samples for LC-MRM-MS analysis**
   1. After IMAC enrichment, resuspend dried samples by adding 20 uL of 3% acetonitrile/5% formic acid to each autosampler vial.
   2. 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 injected by order of increasing concentration, point 1 to point 8, one complete curve followed by the next replicate, with blanks injections and carryover injections performed between each curve. Refer to Table 3 for the complete Run Order.

Table 3. Injection run order of replicate curves

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Curve 1 | |  | Curve 2 | |  | Curve 3 | |
| inj # | Sample |  | inj # | Sample |  | inj # | Sample |
| 1 | michrom\_50fmol\_01 |  | 24 | michrom\_50fmol\_03 |  | 47 | michrom\_50fmol\_05 |
| 2 | blank\_01 |  | 25 | blank\_05 |  | 48 | blank\_09 |
| 3 | blank\_02 |  | 26 | blank\_06 |  | 49 | blank\_10 |
| 4 | blank\_03 |  | 27 | blank\_07 |  | 50 | blank\_11 |
| 5 | pt1\_0fmol\_01\_01 |  | 28 | pt1\_0fmol\_02\_01 |  | 51 | pt1\_0fmol\_03\_01 |
| 6 | pt1\_0fmol\_01\_02 |  | 29 | pt1\_0fmol\_02\_02 |  | 52 | pt1\_0fmol\_03\_02 |
| 7 | pt1\_0fmol\_01\_03 |  | 30 | pt1\_0fmol\_02\_03 |  | 53 | pt1\_0fmol\_03\_03 |
| 8 | pt1\_0fmol\_01\_04 |  | 31 | pt1\_0fmol\_02\_04 |  | 54 | pt1\_0fmol\_03\_04 |
| 9 | pt1\_0fmol\_01\_05 |  | 32 | pt1\_0fmol\_02\_05 |  | 55 | pt1\_0fmol\_03\_05 |
| 10 | pt2\_0002fmol\_01\_01 |  | 33 | pt2\_0002fmol\_02\_01 |  | 56 | pt2\_0002fmol\_03\_01 |
| 11 | pt3\_001fmol\_01\_01 |  | 34 | pt3\_001fmol\_02\_01 |  | 57 | pt3\_001fmol\_03\_01 |
| 12 | pt4\_004fmol\_01\_01 |  | 35 | pt4\_004fmol\_02\_01 |  | 58 | pt4\_004fmol\_03\_01 |
| 13 | pt5\_016fmol\_01\_01 |  | 36 | pt5\_016fmol\_02\_01 |  | 59 | pt5\_016fmol\_03\_01 |
| 14 | pt6\_063fmol\_01\_01 |  | 37 | pt6\_063fmol\_02\_01 |  | 60 | pt6\_063fmol\_03\_01 |
| 15 | pt7\_205fmol\_01\_01 |  | 38 | pt7\_205fmol\_02\_01 |  | 61 | pt7\_205fmol\_03\_01 |
| 16 | pt8\_10fmol\_01\_01 |  | 39 | pt8\_10fmol\_02\_01 |  | 62 | pt8\_10fmol\_03\_01 |
| 17 | carryover\_blank\_01 |  | 40 | carryover\_blank\_03 |  | 63 | carryover\_blank\_05 |
| 18 | carryover\_blank\_02 |  | 41 | carryover\_blank\_04 |  | 64 | carryover\_blank\_06 |
| 19 | wash\_01 |  | 42 | wash\_04 |  | 65 | wash\_07 |
| 20 | wash\_02 |  | 43 | wash\_05 |  | 66 | wash\_08 |
| 21 | wash\_03 |  | 44 | wash\_06 |  | 67 | wash\_09 |
| 22 | blank\_04 |  | 45 | blank\_08 |  | 68 | blank\_12 |
| 23 | michrom\_50fmol\_02 |  | 46 | michrom\_50fmol\_04 |  | 69 | michrom\_50fmol\_06 |

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

***for automated IMAC procedure on AssayMAP Bravo***

1. Abelin, et al. Mol Cell Proteomics. 2016 May;15(5):1622-41. doi: 10.1074/mcp.M116.058354. Epub 2016 Feb 24. PMID:26912667

# 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 |