PRG2009

Sample Preparation of PRG Samples A, B, C, D, E, and F.

1. 200 µl of 1X Seppro® Dilution Buffer (DB10-200, 081021) was added to each of the tubes containing the six samples. The Dilution Buffer contains 10 mM Tris-HCl, 150 mM NaCl, pH 7.4.
2. All the samples were vortexed for 15 – 20 minutes, until the entire dried sample for each tube dissolved into the buffer.
3. Each sample was then loaded into separate Waters sample vials and placed into the auto-sampler of the Waters 2695 Separation Module.
4. The Seppro LC2 IgY14 Column (28-288-12014-LC2) was attached to the Waters Separation Module.
5. The 200 µl samples were then depleted following the protocol supplied by Genway in the Pre-Packed Seppro IgY14 Liquid Chromatography (LC) Column and Starter Reagent Kit. The total run for each sample lasted 50 minutes, and the depleted protein fraction was collected.
6. The samples were then immediately frozen and stored overnight.
7. The following day the samples were thawed and a protein quantification BCA using BSA as the standard curve was preformed.
8. The samples then went through a buffer exchange with 50 mM ammonium bicarbonate, using 3,000 MWCO Amicon Ultra – 4.
9. Following the 1,000 fold buffer exchange the six samples were dried down in an Eppendorf Vacufuge.
10. To each sample 14 µl of dH2O and 14 µl of Laemmli 2X concentrate sample buffer (S3401, 067K6101) was added.
11. A standards solution was prepared using P4649, C6572, and P3235.
12. Two 4 – 20% Tris-HCl, 1.0 mm Criterion™ Pre-cast Gels (C052808B1) were loaded. The gels were run at 150 volts for 90 minutes.
13. The gels were then stained with EZBlue from Sigma-Aldrich (G1041, 058K4362) for 2 hours and destained with dH20 overnight.
14. Gel bands were cut corresponding to the position of the standard proteins. At approximately 100kDa for PYGM and at 30kDa for KLK3 and CGHB.
15. The protein were reduced with TCEP and alkylated with Iodoacetamide and digested overnight with trypsin.
16. The peptides were extracted with 3 times 50 ul of 50% ACN 0.1%TFA.
17. The peptides solution was brought to dryness in a Speed-Vac and resuspended in 50 ul of 0.1%FA.
18. 5ul was injected on the column.

MS analysis.

The samples were run on a LTQ-FT mass spectrometer with a nanospray interface. A 0.1mm Magic C18 column using a 110minute gradient from 8to 38% B. The data was collected in Data dependant mode.

The data was analyzed with Mascot searching Mammals with the SwissProt database and organized with Scaffold.

Quantification was calculated as the ratio of the sum of the XIC's for several peptides (4 for PYGM, 5 for CGHB, 1 for KLK3) and normalized to the sum of the XIC for ITIH4 for PYGM and APOA4 for KLK3 and CGHB.