

## **IMAC enrichment protocol**

(Ficarro SB, et al. 2002. Nature Biotechnology. 20(3): 301.)

### **I. Conversion of carboxylic acid groups to methyl esters**

- Begin with ~20-75pmol protein that has been treated with DTT/Iodoacetamide and digested with appropriate enzyme (see “Sample Preparation: a. Reduction/alkylation with DTT/Iodoacetamide and b. Protease digestion”).
- Dry down digested sample via speed vac until no liquid remains in the Eppendorf tube.
- Make up a solution of 2M methanolic HCl: (add 160µL of acetyl chloride dropwise to 1mL of anhydrous methanol—let sit for 5 minutes).
- Add 30-40µL of 2M methanolic HCl to protein sample.
- Let reaction proceed at room temperature for approximately 2 hours.
- Dry down sample via speed vac until no liquid remains in the Eppendorf tube.
- Reconstitute sample in 1:1:1 mixture of acetonitrile, methanol, and water.

### **II. Preparation of IMAC enrichment column**

- Pack a 360x100µm fused silica column (Polymicro Technologies, Phoenix, AZ) with 8cm of POROS 20 MC packing material (Applied Biosystems, Foster City, CA).
- Activate packing material with 200µL of 100mM FeCl<sub>3</sub> (Aldrich, Milwaukee, WI).
- Load peptide sample onto IMAC column.
- Wash non-specific peptides off column using a solution of 100mM NaCl in 25:74:1 acetonitrile, water, glacial acetic acid.
- Elute phosphorylated peptides off IMAC column onto a pre-column (360x100µm fused silica capillary packed with 6cm of 5-20µm C18 packing material) using 10µL of 50mM Na<sub>2</sub>HPO<sub>4</sub> (columns are butted together with Teflon tubing).
- Rinse pre-column with several column volumes of 0.1% acetic acid to remove Na<sub>2</sub>HPO<sub>4</sub> solution.
- Connect pre-column to analytical column (360x100µm fused silica capillary packed with 6-8cm of 5µm C18 packing material) using Teflon tubing.
- Elute phosphopeptides from pre-column to analytical column and into the mass spectrometer (see “LC-MS analysis” section) for analysis.