

## TiO<sub>2</sub> phosphopeptides enrichment

Protein tryptic digestion and peptides HLB clean-up must be done prior to phosphopeptides enrichment.

1. Resolubilize 307mg of 2,5-Dihydroxybenzoic acid (DHB) with 1.1 mL of 80%ACN/3%TFA (solution A)
2. Resuspend 28mg TiO<sub>2</sub> beads in 138,5uL of solution A
3. Resolubilize 2mg of protein tryptic digest with 200uL of solution A and add 20uL of TiO<sub>2</sub> suspension (ratio 1:2)
4. Incubation of 30min at room temperature with agitation
5. Centrifuge at 5000g for 1min and discard the supernatant
6. Resuspend the beads with 75uL of 30%ACN/3%TFA and transfer the beads into a stage tips, C8, 200uL tip
7. Wash the beads 3 times with 75uL of 30%ACN/3%TFA
8. Wash the beads 3 times with 75uL of 80%ACN/0,3%TFA
9. Elute the phosphopeptides 2 times with 75uL of 40%ACN/17% (NH<sub>4</sub>)OH
10. Dry the eluate using a speed-vac