Protocol for phosphoenrichment with TiO_2 beads

TiO_2 beads are from GL Sciences (Titansphere TiO, 5 um, 500 mg, 5020-75000)

Adapted from JA Paulo et al, Proteomics, 2015, 15, 462-473

Preparation of solutions

2M lactic acid/50% ACN

Note: Lactic acid: 90.08 g/mol, density 1.209 g/mL, 85% purity. Molarity of this solution is 11.3 M.

Mix 16.2 mL water, 25 mL ACN and 8.8 mL concentrated (11.3 M) lactic acid

50 mM K_2HPO_4 pH 10

Add 218 mg of K_2HPO_4 (MW=174.18 g/mol) to 20 mL of water. Adjust to pH 10 with NH_4OH, then adjust volume to 25 mL.

Procedure

1. Resuspend peptides in 900 uL of 2M lactic acid/50% ACN. Vortex for 15 min at RT.
2. Weigh out enough beads for a 4:1 bead: peptide ratio. After washing the beads (see below), prepare a solution of ~0.33 mg/uL using 2M lactic acid/50% ACN, keeping in mind that the spun down TiO2 beads account for ~1/4 of the volume. (For example, 400 mg of beads spins down to ~300 uL, so add 900 uL of 2M lactic acid/50% ACN to make 1200 uL total).

Step	Solution	Volume (uL)	Conditions
TiO_2 Beads			
Wash 3x	2M lactic acid/50% ACN	1000	Vortex 15 s, centrifuge 30 s
Prepare 0.33 mg/uL solution (see above). Add beads to peptide mixture using a 4:1 bead:peptide ratio.			
Rotate	Bead:peptide mixture	~1000	1 h at RT, then centrifuge 30 s. Remove supernatant*
Wash 2x**	2M lactic acid/50% ACN	500	Vortex 15 s, centrifuge 30 s
Wash 2x**	0.1% TFA/50% ACN	500	Vortex 15 s, centrifuge 30 s
Wash 2x**	0.1% TFA/25% ACN	500	Vortex 15 s, centrifuge 30 s
Elute 2x	50 mM K_2HPO_4 pH 10	200	Vortex 5 min, centrifuge 30 s
Speed vac the extracts to dryness immediately as they might not be stable at pH 10			

Centrifuge steps should be at high speed

*The supernatant can be saved for other enrichments, or as a precaution

**The washes can be saved as a precaution