Protocol for Cell Signaling PTMScan Ab enrichment (Immunoaffinity Purification, IAP)

Product	Part no.
PTMScan Pan-Methyl Lysine Immunoaffinity beads	14722
PTMScan Acetyl-Lysine Motif [Ac-K] Immunoaffinity beads	13416
PTMScan® Phospho-Tyrosine Motif (Y*) (P-Tyr-1000) Immunoaffinity Beads	8803

Adapted from Martinez-Val et al, Journal of Proteome Research, 2017

1. Dilute the 10x IAP buffer to 1x with water.
2. Resuspend peptides in 1 mL of 1x IAP buffer. Vortex for 15 min at RT. Test the pH to ensure the pH is ~ 7.

Step	Solution	Volume (uL)	Conditions
80 uL of Ab slurry (IAP beads)			
Wash 3x	PBS (50 mM Na_2HPO_4, 150 mM NaCl)	500	Invert several times, centrifuge 30 s
Wash 1x	1x IAP	400	Invert several times, centrifuge 30 s
Option 1, one tube/sample: Add resuspended peptide mixture to spun down Ab slurry Option 2, one tube/2 samples: Add 300 uL IAP to the washed slurry, then pipette half (~160 uL) into each tube			
Rotate	Peptide:slurry mixture	~1000	4C for 2 h. Centrifuge 30 s and remove the supernatant*
Keep the wash solutions on ice			
Wash 2x**	1x IAP	500	Invert several times, centrifuge 30 s
Wash 3x**	water	500	Invert several times, centrifuge 30 s
Elute 2x	0.15% trifluoroacetic acid	50	Agitation at RT, 10 min
Centrifuge to remove any remaining beads, transfer supernatant to a new tube, speed vac and/or proceed to desalting step using the Nest Group spin tips.			

Centrifuge steps should be at low speed (2000 rcf or g)

*The supernatant can be saved for subsequent analyses

**The washes can be saved as a precaution