Protocol for peptide desalting using NestGroup Spin columns

Adapted from the UW Proteomics protocol and Cell Signaling protocol

Solvent	Most applications	PTM Scan Ab enrichment
A (Condition)	80% ACN, 0.1% TFA	50% ACN, 0.1% TFA
B (Equilibrate/wash)	0-5% ACN, 0.1%TFA	0-5% ACN, 0.1%TFA
C (Elution)	80% ACN, 0.1% FA	40% ACN, 0.1% TFA

Column	Protein Capacity (ug)	Loading volume (uL)	Part no.
UltraMicroSpin	3-30	2-100	SUM SS18V
MicroSpin	5-60	5-200	SEM SS18V
BioPure SPN MIDI	17-170	25-100	HEM S18V
MacroSpin	30-300	50-450	SMM SS18V

Spin each step for 2 min at 2000 rpm (~800 rpm on an Eppendorf mini centrifuge)

Step/Solvent	# repeats	UltraMicroSpin	MicroSpin	BioPure SPN MIDI	MacroSpin
Condition with A	3/4*	100	100	100	500
Equilibrate with B	3/4*	50	100	100	500
Load sample	3	All	All	All	All
Wash with B	3	25	50	75	250
	Place column in a new centrifuge tube to collect eluate				
Elute with C	3/4*	50	50	50	250

*four repeats are required for some steps when using the BioPure SPN MIDI columns

After elution, part of the sample may be set aside for subsequent peptide and/or verification assays (eg- verify that a phosphoenrichment worked).

After elution, the samples are speed vacuumed to dryness.