General info

Protocol information

Protocol name MagReSyn_MOAC_phosphopeptide_enrichment_KF_Flex

Modified by SSTOYCHEV

Kit name Phosphopeptide Enrichment

Description KingFisher Flex protocol for enrichment of phosphopeptides (Tape et al.,

2014)

Example shown is for enrichment of 200ug digest with MagReSyn TiO2

or ZrO2 microparticles

Reagent info

P1 Microspheres 96 DW plate				
Name TiO2 (25mg/ml) or ZrO2	Well volume [µl]	Total reagent volume [μl]	Type	
(50mg/ml)	80	-	Reagent	
80% ACN, 5% TFA, 1M GA (bind buffer)	170	-	Reagent	
P2 Microsphere Wash		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Type	
80% ACN, 5% TFA, 1M GA	500	-	Reagent	
P3 Sample		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Type	
200ug digest in 80% ACN 5% TFA, 1M GA	, 200	-	Sample	
P4 Wash 1		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Type	
80% ACN, 5% TFA, 1M GA	500	-	Reagent	
P5 Wash 2		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Type	
80% ACN, 1% TFA	500	-	Reagent	
P6 Wash 3		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Туре	
10% ACN, 0.2% TFA	500	-	Reagent	
P7 Elution		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Туре	
1-2% NH4OH	200	-	Reagent	
Tip Plate		96 DW plate		
Name	Well volume [μl]	Total reagent volume [μl]	Type	
-	-	-	-	

Steps data

	Tip1		96 DW tip comb	
	9	Pick-Up	Tip Plate	
	<mark>.</mark>	CollectBeads1	P1 Microspheres Collect count Collect time [s]	3 10
	es°	Equilibration	P2 Microsphere Wash	
		Beginning of step Mixing / heating: End of step	Precollect Release time, speed Mixing time, speed Heating during mixing Postmix Collect count	No 00:00:05, Medium 00:05:00, Medium No No 3
			Collect time [s]	2
	�	p-peptide bind	P3 Sample	
		Beginning of step Mixing / heating: End of step	Precollect Release time, speed Mixing time, speed Heating during mixing Postmix Collect count Collect time [s]	No 00:00:05, Medium 00:20:00, Medium No No 3
	$\stackrel{\circ}{\sim}$	Wash 1	P4 Wash 1	
		Beginning of step Mixing / heating: End of step	Precollect Release time, speed Mixing time, speed Heating during mixing Postmix Collect count Collect time [s]	No 00:00:05, Medium 00:02:00, Medium No No 3 2
	$\stackrel{\sim}{\simeq}$	Wash 2	P5 Wash 2	
		Beginning of step Mixing / heating: End of step	Precollect Release time, speed Mixing time, speed Heating during mixing Postmix Collect count Collect time [s]	No 00:00:05, Medium 00:02:00, Medium No No 3 2

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e ²	Wash 3	P6 Wash 3	
	Beginning of step	Precollect Release time, speed	No 00:00:05, Medium
	Mixing / heating:	Mixing time, speed Heating during mixing	00:02:00, Medium No
	End of step	Postmix Collect count Collect time [s]	No 3 2
	Elute	P7 Elution	
	Beginning of step	Precollect Release time, speed	No 00:00:05, Medium
	Mixing / heating:	Mixing time, speed Heating during mixing	00:10:00, Medium No
	End of step	Postmix Collect count Collect time [s]	No 3 2
<u></u>	ReleaseBeads1	P1 Microspheres	
		Release time, speed	00:00:10, Fast
9	Leave	Tip Plate	