General info

Protocol information

Protocol name MagReSyn_IMAC_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 Ti-

IMAC or Zr-IMAC microparticles

Reagent info

Name Well volume [μl] Total reagent volume [μl] Type Ti/Zr-IMAC (20mg/ml) in storage (20% EtO 40 - Reagent 80% ACN, 5% TFA, 0.1M GA (bind buffer) 160 - Reagent P2 Microsphere Wash 96 DW plate Name Well volume [μl] Total reagent volume [μl] Type			
GA (bind buffer) P2 Microsphere Wash Name Well volume [μl] Name [μl] Total reagent volume [μl] Type			
Name Well volume [µl] Total reagent volume [µl] Type			
80% ACN, 5% TFA, 0.1M 500 - Reagent			
P3 Sample 96 DW plate			
Name Well volume [µl] Total reagent volume [µl] Type			
200ug digest in 80% ACN, 200 - Sample 5% TFA, 0.1M GA			
P4 Wash 1 96 DW plate			
Name Well volume [µl] Total reagent volume [µl] Type			
80% ACN, 5% TFA, 0.1M 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] Type			
10% ACN, 0.2% TFA 500 - Reagent			
P7 Elution 96 DW plate			
NameWell volume [μl]Total reagent volume [μl]Type1-2% NH4OH200-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	
	- Good	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 Collect time [s]	No 00:00:05, Medium 00:05:00, Medium No No 3 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
	$\stackrel{\circ}{\simeq}$	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, Fast No No 3
	$\stackrel{\circ}{\simeq}$	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, Fast No No 3

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	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, Fast 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
	ReleaseBeads1	P1 Microspheres	
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-00		Release time, speed	00:00:10, Fast