

**Protocol report**

MagReSyn\_HILIC\_Protein\_capture\_clean\_digest\_KF\_Flex

01/03/2021 09:50+02:00

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**General info****Protocol information**

Protocol name	MagReSyn_HILIC_Protein_capture_clean_digest_KF_Flex
Modified by	SSTOYCHEV
Kit name	HILIC clean-up
Description	KingFisher Flex 96 protocol for on-bead prortein capture, clean-up and digestion using MagReSyn HILIC magnetic microspheres. Example shown is for 50ug lysate and 2hr digest

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**Reagent info**

<b>P1 Microspheres</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
HILIC Microspheres (20mg/ml)	25	-	Reagent	
Bind buffer 15% ACN,100mM Acetate pH 4.5	125	-	Reagent	

<b>P2 Microsphere Equil</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
Bind buffer 15% ACN,100mM Acetate pH 4.5	500	-	Reagent	

<b>P3 Protein bind</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
Lysate: 50ug in 1-5% SDS, 50mM Tris pH 8	100	-	Sample	
Bind buffer 30% ACN, 200mM Acetate p4.5	100	-	Reagent	

<b>P4 Wash 1</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
95% ACN	500	-	Reagent	

<b>P5 Wash 2</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
95% ACN	500	-	Reagent	



  

<b>P8 On Bead Digest</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
50mM Amm Bic pH 8	250	-	Reagent	
LysC/ Trp (or Trp only 1:20 for 2hr dige	2	-	Reagent	

<b>Tip Plate</b>		<b>96 DW plate</b>		
<b>Name</b>	<b>Well volume [µl]</b>	<b>Total reagent volume [µl]</b>	<b>Type</b>	
-	-	-	-	

## Steps data




	Tip1	96 DW tip comb	
	Pick-Up	Tip Plate	
	CollectBeads1	P1 Microspheres	
		Collect count	3
		Collect time [s]	15
	Equilibrate Microspheres	P2 Microsphere Equil	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Medium
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	15
	Bind Protein	P3 Protein bind	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Medium
	Mixing / heating:	Mixing time, speed	00:30:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	15
	Wash 1	P4 Wash 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Medium
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	15
	Wash 2	P5 Wash 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Medium
	Mixing / heating:	Mixing time, speed	00:01:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	15

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	Digest	P8 On Bead Digest	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Medium
	Mixing / heating:	Shake 1 time, speed	00:00:15, Medium
		Shake 2 time, speed	00:02:15, Paused
		Loop count	52
		Tip position when paused	Tip edge in liquid
		Heating temperature [°C]	47
	End of step	Preheat	No
		Postmix	No
		Collect count	3
		Collect time [s]	15
	ReleaseBeads1	P1 Microspheres	
		Release time, speed	00:00:30, Medium
	Leave	Tip Plate	