





Jun 08, 2022

S Binding of Rab29 to the LRRK2 Armadillo domain by Microscale Thermophoresis

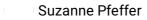
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Microscale thermophoresis (MST) is a powerful tool to measure the affinities of interactions between proteins. We present here our method for determining the binding of Rab29 GTPase to the LRRK2 (Leucine rich repeat kinase 2) N-terminal Armadillo domain. Work from several labs has shown that Rab29 can recruit LRRK2 to the Golgi, where it normally resides, or to other compartments, when artificially relocalized to another cellular compartment. MST has enabled us to define the precise binding site for Rab GTPases on the LRRK2 Armadillo domain.

DOI

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ASAPCRN protocol ,

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Eppendorf LoBind tubes 0.5mL Catalog No. 0030108434

Eppendorf LoRetention tips 0.1-10uL Catalog No. 022493018

pET21b HisSumo Rab29 full length wild type (tag cleaved off)

Buffer exchange isolated Proteins

- 1 Buffer: 50mM Hepes pH 8, 100mM NaCl, 5mM MgCl₂, 20μM GTP, 0.2mM TCEP, 5% glycerol
 - 1. Use either a Nanotemper "A-Column" provided in labeling kit or (our preference) 0.5 ml Zeba Spin column 7MWCO. Twist off bottom of column and loosen cap.
 - 2. Prespin column: Centrifuge at 4°C 1500xG for 1 min to remove storage solution.
 - 3. Apply 300µL of buffer to center of resin bed and centrifuge at 1500xG for 1min. Do this 4X.
 - 4. Place column in fresh collection tube. Apply $100\mu L$ of your sample to resin and spin at 1500xG for 2min.
 - 5. Protein is in collected flowthrough.

2

2.1 0.

- 3 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
- 4 Arial'>
- 5 font-family:Arial'>Labeling is done with 2nd Generation NHS RED
- 6 label from Nanotemper

6.1 1.

protocols.io

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7	'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
8	Arial'>
9	font-family:Arial'>Dye is resuspended in 25µl DMSO as per protocol from Nanotemper
10	to make it 600µM final stock concentration
	10.1 2.
11	'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
12	Arial'>
13	font-family:Arial'>Set up 100µL labeling reaction using a 3:1 ratio of dye to
14	protein; Dye is used at final concentration of 30µM; buffer exchanged Armadillo
15	is used at a final concentration of 10μM (note: reducing reagent will interfere

16 with labeling. Low concentration TCEP is acceptable) **16.1** 3. 17 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family: 18 Arial'> 19 font-family:Arial'>Add above buffer to bring volume to 100µL final volume, mix 20 by flicking tube 20.1 4. 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family: 21 22 Arial'> 23 font-family: Arial' > Incubate for 30min in dark at room temperature 23.1 5.

24	'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
25	Arial'>
26	font-family:Arial'>Desalt excess dye using column B provided in kit or another
27	Zeba Spin column as before
	27.1 6.
28	'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
29	Arial'>
30	font-family:Arial'>Spin labeled sample hard 14000xg for 10min at 4°C to remove
31	any aggregates
	31.1 7.

32	'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:
33	Arial'>
34	font-family:Arial'>Take absorbance 280nm of labeled protein and use extinction
35	coefficient and the Nanotemper Degree of Labeling (DOL) calculator to determine
36	concentration and DOL https://nanotempertech.com/dol-calculator/. DOL should be
37	between 0.5-1
38	Normal
39	0
40	false
41	false
42 S proto	false 6

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44	JA
45	X-NONE
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52	mso-style-priority:99;

53	mso-style-parent:"";
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66	mso-hansi-theme-font:minor-latin;}

68 of LRRK2 Armadillo

Labeling

67