



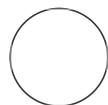
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🌐 Microscopy-based bead protein-protein interaction assay

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Protocol status: Working
 We use this protocol and it's working

Created: Jun 07, 2023

ABSTRACT

This protocol describes how to perform microscopy-based bead protein-protein interaction assay with GST- or mCherry-tagged proteins as baits and fluorescently-tagged proteins as preys. The protocol requires to have purified proteins and allows to monitor protein-protein interaction in an equilibrium state. The fluorescent signal can be quantified.

GUIDELINES

Experiment should be repeated at least three times for statistical analysis.

MATERIALS

Glutathione Sepharose 4B (Cytiva)
 RFP-Trap Agarose beads (ChromoTek)
 384-well glass-bottom microplate (Greiner Bio-One)

confocal microscope
 ImageJ software

Last Modified: Jul 31, 2023

BEFORE START INSTRUCTIONS

PROTOCOL integer ID:
83013

SEC Buffer:
25 mM HEPES pH 7.5
150 mM NaCl
Freshly added: 1 mM DTT

Keywords: ASAPCRN

Purify tagged bait and prey proteins

Protocol



NAME

EXPRESSION AND PURIFICATION OF HUMAN NEMO (GST-GFP-NEMO)



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PREVIEW




Prepare bait-coated beads

1h 2m



- 1 Equilibrate  20 μL Glutathione Sepharose 4B or RFP-Trap Agarose beads with  200 μL SEC buffer

Note


Prepare these bead equilibrations for each condition.

- 2 Incubate equilibrated beads with GST- or mCherry-tagged bait protein for a final concentration of  5 micromolar (μM) in SEC buffer for  01:00:00 at  4 $^{\circ}\text{C}$ with gentle rotation.


1h

- 3 Centrifuge beads at 3000 rcf for  00:02:00 at  4 $^{\circ}\text{C}$

2m

4 Remove the supernatant and wash beads with  200 μL SEC buffer

4.1 Repeat for a total of 2 washes, then discard buffer


5 Add  20 μL SEC buffer to achieve a beads:buffer ratio of 1:1

Interaction assay set-up



30m

6 Pipette prey proteins into the wells of a 384-well glass-bottom microplate (Greiner Bio-One)

Note


A volume of at least  20 μL should be used to cover the bottom of the well



Note

Prey concentration should be  0.1 micromolar (μM) to  1 micromolar (μM), but should be adjusted depending on the strength of the interaction

Note

Different preys should be conjugated to different fluorophores

7 Pipette  1 μL of bait-coated beads into each well


8 Incubate the plate for  00:30:00 in the dark at  Room temperature 30m

Signal detection

- 9 Use a microscope configured to detect fluorescent signal (e.g. Zeiss LSM 700 confocal microscope equipped with Plan-Apochromat 20X/0.8 objective)
- 10 Acquire fluorescent images in the middle section of the beads and collect more than one image for each well
- 11 Also acquire bright field images for each field

Quantification using ImageJ

- 12 Draw several lines across each bead in the fluorescent channel and measure the intensity along the lines
- 13 Record the maximum intensity for each bead
- 14 For background correction, measure the average intensity of a rectangular ROI that covers an area of each field of view with no beads
- 15 Subtract the average intensity of the background ROI from each bead maximum in that field

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- 16** Calculate the average of the background-corrected maximum intensities of beads for each sample