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

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Microscopy-based bead protein-protein interaction assay

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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

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BEFORE START INSTRUCTIONS

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

Purify tagged bait and prey proteins

Protocol

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

CREATED BY
OLIVIA HARDING

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 [M] 5 micromolar (µM) in SEC buffer for 01:00:00 at 4 °C with gentle rotation. 1h

3 Centrifuge beads at 3000 rcf for 00:02:00 at 4 °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 [M] 0.1 micromolar (µM) to [M] 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

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