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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-labelled proteins as baits and fluorescently labelled 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.

ATTACHMENTS

beads assay.pdf

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PROTOCOL CITATION

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MATERIALS TEXT

Pull down buffer: 25mM TRIS-Cl pH 7.5, 150mM NaCl, 1mM DTT
Glutathione Sepharose 4B (Cytiva)
384-well glass-bottom microplate (Greiner Bio-One)

purified GST-labeled bait proteins
purified fluorescently-labeled prey proteins

confocal microscope
ImageJ software

Bait preparation

1 Wash 20 µl of Glutathione Sepharose 4B (Cytiva) beads slurry with 200 µl distilled water and with 200 µl of pulldown buffer (e.g. 25mM TRIS-Cl pH 7.5, 150mM NaCl, 1mM DTT).

2 Mix the beads with a GST-labelled bait protein at the final concentration between 1 - 10 µM in a pulldown buffer in a 0.5 ml Eppendorf tube. Incubate for at 4 °C for 1 h gently rolling or rocking.

3 Spin down the beads (4000 rpm, 2 min, 4 °C) and remove the supernatant. Wash the beads twice with 200 µl of pull-down buffer and finally add 20 µl of pull-down buffer to have 1:1 beads:buffer ratio.

Interaction assay

4 Pipette the preys onto 384-well glass-bottom microplate (Greiner Bio-One). The minimal volume to cover the bottom of a well is 20 µl. The final concentration of the prey may vary depending on the strength of the interaction, usually between 0.1-1 µM. The number of proteins in the well can be adjusted to the experimental set-up. Importantly, different preys should have different flourophores attached for detection (e.g.prey1-eGFP and prey2-mCherry).

5 Pipette 1 µl of bait-coated beads to each well. Incubate the plate for 30 min in the dark at room temperature. Do not apply rocking or mixing.

Signal detection

Image the beads in the wells with a microscope configured to detect fluorescent signal. When

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6 imaging beads for further quantification it is recommended to use a confocal microscope and to adjust the focus to take the plane in the middle of the beads. As the size of the beads vary it may be necessary to acquire more than one image of one field of view. For a reference also collect bright field images.

Signal quantification

7 For signal quantification, in ImageJ software draw eight lines across each bead that is in focus. Take the maximum brightness value along each the line that was taken (see attached pdf file).

8 To correct for background fluorescence draw a small rectangle that covers an empty area (beads-free) of each picture. Take the average (not maximum) brightness of that area and subtract from the maximal fluorescence for each bead from that image.

9 Calculate an average of the corrected maximum intensities of all the beads to get the final value of the signal intensity of the replicate. Repeat the same experiment at least three times for statistical analysis.

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