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Microscopy-based bead protein-protein interaction assay

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ABSTRACT

An assay to study protein-protein interaction

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- 1 Wash glutathione sepharose beads (GE Healthcare) with the reaction buffer (20 mM HEPES at pH 8.0, 200 mM NaCl and 1 mM TCEP) three times.
- 2 Make a mixture of 0.5 μ M GST or GST tagged cargo receptors and different ATG proteins.
- 3 Incubate the protein mixture with 10 μ L washed glutathione sepharose beads at room temperature for 1 h.
- 4 Wash the beads three times with reaction buffer.

- 5 Suspend the beads with 120 μ L reaction buffer and transfer the beads to the observation chamber.
- 6 Acquire images on a Nikon A1 confocal microscope with a 63 \times Plan Apochromat 1.4 NA objective.