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In Situ Pull-down Protein Interaction Analysis

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Katja Piltti[1], Zeina Elrachid[1]

[1]University of California, Irvine

Katja Piltti
University of California, Irvine

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We use this protocol and it's working

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Abstract

An *in situ* His-tag pull-down assay to test direct interactions between C1q, BAI1, and p32 demonstrates direct binding of C1q–BAI1, C1q–p32, and BAI1–p32, and that C1q-BAI1-p32 can be pulled down as a complex.

Materials

- His-tag recombinant human BAI1 (SinoBiological, 4969-BA)
- His-tag p32 (SinoBiological, 11874-H08E)
- Magnetic beads (Invitrogen, 10103D)
- Purified human C1q (MyBioSource, MBS147305)
- Recombinant untagged human p32 (LSBio, LS-G3375-20)
- Binding/wash buffer (50 mM sodium phosphate, 300 mM NaCl, 0.01% Tween-20)
- Pulldown buffer (3.25 mM sodium phosphate, 70 mM NaCl, 0.01% Tween-20)
- His-elution buffer (300 mM imidazole, 50 mM sodium phosphate, 300 mM NaCl, 0.005% Tween-20)

Prepare Bait Proteins

1 Dissolve 5 µg of His-tag recombinant human BAI1 (SinoBiological, 4969-BA) and His-tag p32 (SinoBiological, 11874-H08E) in 1x binding/wash buffer (50 mM sodium phosphate, 300 mM NaCl, 0.01% Tween-20).

2 For each pull-down, immobilize the bait proteins on 2 mg of magnetic beads (Invitrogen, 10103D) in Eppendorf tube(s) by incubating on a rocker for 10 minutes at room temperature (RT).

Collect Excess Bait

3 Place tubes on a magnet (Invitrogen, 12321D) to separate beads from the buffer.

4 Collect the buffer containing excess bait for Western blot validation.

Wash Beads

5 Wash the beads four times with 1x binding/wash buffer to remove any remaining excess bait protein.

Prepare Prey Proteins

6 Dissolve 5 µg of purified human C1q (MyBioSource, MBS147305) and recombinant untagged human p32 (LSBio, LS-G3375-20) in 1x pulldown buffer (3.25 mM sodium phosphate, 70 mM NaCl, 0.01% Tween-20).

Incubate Prey with Bait

7 Incubate the single prey proteins or combination of the prey proteins with the magnetic bead-immobilized bait in the Eppendorf tube(s) on a rocker for 15 minutes at RT.

Capture Protein Complexes

8 Use a magnet to separate the beads and wash four times with 1x binding/wash buffer to remove unbound prey proteins.

Elute Proteins

9 Elute the bound prey and bait using his-elution buffer (300 mM imidazole, 50 mM sodium phosphate, 300 mM NaCl, 0.005% Tween-20).

10 Analyze the eluted samples using Western blotting to confirm interactions.