Co-Streptavidin Precipitation

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[Abstract] Co-Streptavidin Precipitation (Co-SP) is a method to pull down protein partners of a protein of interest tagged with the streptavidin binding protein domain, and using streptavidin columns that specifically bind to Streptavidin Binding Protein (SBP) in order to test the protein-protein interactions. Proteins of interest to be tested for their interaction are artificially co-expressed in *"easy to transfect"* cells. Pull down proteins can be analyzed by western blot for suspected protein partner.

Materials and Reagents

1. HeLa cells (ATCC, catalog number: CCL-2)
2. SBP-Calmodulin Tag Expression Vector pNTAP (Inter Play N-Terminal Mammalian TAP Vector, Agilent Technologies-Stratagene, catalog number: 240101)
3. Lipofectamine (Life Technologies, Invitrogen™, catalog number: 11668-027)
4. Fugene 6 (F. Hoffmann-La Roche, catalog number: 05061377001)
5. FLAG tag Expression Vector pCMV-Tag2 (Agilent Technologies-Stratagene, catalog number: 211172)
6. NP40
7. Steptavidin agarose beads (Sigma-Aldrich, catalog number: S-1638)
8. Anti- Calmodulin antibody (Upstate, catalog number: 07-482)
9. Anti- Flag antibody (Sigma-Aldrich, catalog number: F1804-200UG)
10. Protease inhibitor cocktail, EDTA free(F. Hoffmann-La Roche, catalog number: 04693159001)
11. DNase I (Sigma-Aldrich, catalog number: DN25)
12. Benzonase nuclease (Sigma-Aldrich, catalog number: E1014)
13. Laemmli loading buffer (see Recipes)
14. Sucrose buffer (see Recipes)
15. Nuclei Lysis buffer for hard to extract nuclear factors (see Recipes)
16. Nuclei Lysis buffer for soluble nuclear factors (see Recipes)

Equipment

1. Wheel in a cold room
2. Refrigerating centrifuge 1.5 ml tubes

Procedures

A. Cell transfection
   1. Co-transfected 10 to 50 x 10^6 of HeLa cells (or other "*easy to transfect cells*") with your gene of interest "1"-FLAG cloned into pCMV-Tag2 expression vector, and your gene of interest "2"-SBP-Calmodulin cloned into pNTAP, or the empty expression vector pNTAP as a control. Transfection is performed with standard transfection protocols (such as Lipofectamine, Invitrogen or Fugene 6, Roche or Calcium Chloride precipitation).

B. Nuclear extract preparation (also see Dadi *et al.*, 2013)
   24 to 48 H after transfection, the cells are lysed and nuclear extracts were prepared using the nuclear extract lysis protocol:
   1. Use trypsin to recover the cells and wash cells with cold 1x PBS then centrifuge for 6 min at 300 *x g* at 4 °C. From now on, all the steps should be performed on ice.
   2. Resuspend the cell pellet in the chilled Sucrose buffer (5 µl/ 1 x 10^6 cells).
   3. Add vol/vol Sucrose buffer containing 0.5% NP40 (final concentration 0.25%).
   4. Mix by pipetting on ice. Save a small aliquot (1 to 5% -aliquot 1).
   5. Centrifuge 10 min at 1,100 *x g* at 4 °C. The pellet contains the nuclei and looks nacreous to white. The supernatant contains the cytoplasmic protein extract and can be saved if a cytoplasmic protein is of interest for Co-IP. Save a small aliquot (1 to 5% -aliquot 2).
   6. Wash the pellet with Sucrose buffer (without NP40) and centrifuge 10 min at 2,000 rpm at 4 °C.
   7. Depending on the nuclear proteins to be purified, you can lyse nuclei with:
      a. Either the Nuclei Lysis buffer for soluble proteins (5 µl/ 1 x 10^6 cells);
      b. Or the Nuclei Lysis buffer for hard-to-extract proteins (5 µl/ 1 x 10^6 cells) and add DNase 5 U/µl and Benzonase 5 U/µl. Resuspension is hard since it's very viscous with DNA.
   8. Incubate on a wheel at 4 °C for 45 min to 1 h. Save a small aliquot (1 to 5% -aliquot 3).
   9. Centrifuge 3 min 10,000 *x g* at 4 °C. The supernatant is the protein nuclei extract that will serve for the IP. Save a small aliquot from the supernatant (1 to 5% -aliquot 4; which is also the input of the IP experiment) and the pellet is the insoluble substance such as

membrane debris. Resuspend the pellet in 5 µl/ 1 x 10^6 cells of 150 mM NaCl, 10 mM Tris. Save a small aliquot (1 to 5% -aliquot 5).

10. Verify the efficiency of the lysis by analyzing by SDS-PAGE and western-blot the presence of your proteins of interest in the saved aliquots.
    i. Aliquot 1: total cells
    ii. Aliquot 2: cytoplasm protein extract
    iii. Aliquot 3: total nuclei extract
    iv. Aliquot 4: nuclear protein extract
    v. Aliquot 5: insoluble nuclei extract

C. Co-streptavidin purification (Refs 2-4)

1. Incubate the protein nuclei extract 2 h at 4 °C with 25 µl of streptavidin agarose (bead volume). Optimization is required for efficient SP of your SBP-protein of interest and the quantity of streptavidin beads may vary according to the protein stability, etc.
2. Wash 4 to 6 times the beads in 100 mM NaCl, 10 mM Tris, HCl pH 7.8, by mixing by pipetting and centrifuging 10,000 *x g* for 30 sec.
3. Elute the bound proteins in Laemmli loading buffer and separate by SDS-PAGE and analyze by western blot using antibodies against Calmodulin and Flag.

Recipes

1. Laemmli buffer
   20 % Glycerol
   4% SDS
   250 mM Tris (pH 6.8) (stacking buffer for upper gel of SDS PAGE)
   1.4 M 2-mercapthoethanol
   A pinch of bromophenol blue
2. Sucrose buffer
   0.32 M Sucrose
   3mM $CaCl_2$
   2mM MgOAc
   0.1 mM EDTA
   10 mM DTT
   0.5 mM PMSF
3. Nuclei Lysis buffer for hard to extract nuclear factors
   50 mM Hepes (pH 7.8)
   3 mM $MgCl_2$

300 mM NaCl
1 mM DTT
0.1 mM PMSF
Protease inhibitor complete mini EDTA free tablets 1x

4. Nuclei Lysis buffer for soluble nuclear factors
   50 mM Hepes（pH 7.8)
   50 mM KCl
   300 mM NaCl
   0.1 mM EDTA
   10 % Glycerol
   1 mM DTT
   0.1 mM PMSF
   Protease inhibitor complete mini EDTA free tablets 1x

Notes

1. The number of cells to be transfected need to be optimized, depending on the size and the stability of the proteins to be expressed.

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