Protocol for Calbindin D28k Antibody (Cat. No. 214 004)
Immunoprecipitation (IP)
Standard Condition

In immunoprecipitation approaches, antibodies are employed to specifically pull down the corresponding target molecule from a complex sample like a tissue or cell lysate. Isolated proteins or complexes can be analyzed further to identify potential interaction partners of the target molecule or to determine the specificity of an antibody.
Tissue or cells have to be solubilized before IP to make the proteins accessible for the antibody.

Materials and reagents

- **Solubilization buffer A:** (20 mM Tris, 50 mM NaCl, 5 mM EDTA, 2% Triton X-100), pH 7.4
- **Washing buffer B:** (10 mM Tris), pH 7.4
- **Washing buffer C:** (10 mM Tris, 150 mM NaCl, 0.2% Triton X-100), pH 7.4
- **Blocking buffer:** (10 mM Tris, 2% BSA), pH 7.4
- **Beads:** Protein A coupled to agarose or sepharose beads
- **Protease Inhibitors:** Commercially available cocktails are recommended.
- **Phosphatase inhibitors:** Commercially available cocktails are recommended.
- **Primary antibody:** Calbindin D28k antibody (cat. no. 214 004)

Procedure

- All steps should be carried out at 4°C. Protein solubilization (solubilization step 1) and binding of the antibodies to the beads (immunoprecipitation step 1) can be carried out in parallel.
- Supplement **solubilization buffer A, washing buffer B, C and blocking buffer** with protease and/or phosphatase innhibitors according to the manufacturer's recommendation.

Solubilization with Triton X-100

1. Solubilize proteins by adding a 1:1 volume of **solubilization buffer A** containing protease and/or phosphatase inhibitors to the cell or tissue sample (final concentration of 5 mM Tris/HCl, 25 mM NaCl, 2.5 mM EDTA, 1% Triton X-100).
   Starting material should have a concentration of 1-4 mg/ml.

2. Centrifuge unsolubilized material at 9600x g for 10 min. The supernatant will be used for IP. If desired, the pellet can be kept for further analysis.

Immunoprecipitation

1. Wash 10 µl **beads** with 100 µl **washing buffer B** for 15 min.

2. Centrifuge **beads** for 5 min at 2400x g and discard the supernatant.

3. Incubate 5 µl antiserum with **beads** in 200 µl **washing buffer B** for 1 h to bind.

4. Centrifuge **beads** for 5 min at 2400x g and discard the supernatant.

5. Block **beads** with 200 µl of **blocking buffer** for 30 min.
6. Centrifuge **beads** for 5 min at 2400x g and discard the supernatant.
7. Wash **beads** with **washing buffer B,** centrifuge beads for 5 min at 2400x g, and carefully remove **washing buffer B.**
8. Add 100-200 µl of the sample and incubate for 1-2 h at 4°C rotating head over tail.
9. Centrifuge **beads** for 5 min at 2400x g and collect supernatant for subsequent analysis.
10. Wash suspension twice with **washing buffer C.** Centrifuge **beads** for 5 min at 2400x g and remove **washing buffer C.**
11. For SDS-PAGE analysis, incubate the pellet with SDS loading buffer and apply to SDS-PAGE. Apply starting material and supernatant from step 7 for comparison.

Remarks

- Some proteins are not efficiently solubilized by Triton X-100. For these proteins, the denaturing solubilization protocol is recommended. For further details have a look at: Geumann C, Grønborg M, Hellwig M, Martens H & Jahn R (2010). A sandwich enzyme-linked immunosorbent assay for the quantification of insoluble membrane and scaffold proteins. Analytical Biochemistry 402: 161-9.
- If membrane proteins are immunoprecipitated, make sure that detergent is included in all steps that contain your target protein.