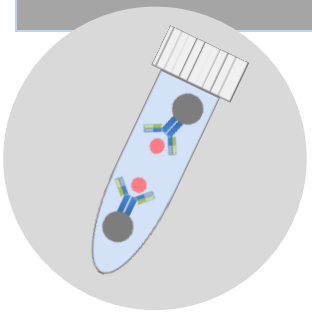
Davids Protocols



Immunoprecipitation – IP for Chicken Antibodies: IgY

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Immunoprecipitation (IP) can be used to purify proteins from a protein mixture utilizing antibodies that bind to the protein of interest. In the first step, the antibody binds the protein in a solution. In a second step the antibody/antigen complex will be precipitated using ProteinA/G material. That has the effect, that the protein of interest will be separated from the remaining sample. Usually this sample is used in WesternBlot or SDS-PAGE afterwards.

IgY do not bind to ProteinA or ProteinG material. For this reason Davids researched a new matrix material that is able to precipitate IgY. It is based on a mouse anti-IgY antibody that is able to bind and precipitate the IgY in one step. Alternatively, the pure mouse anti-IgY antibody can be used to bind the IgY. In a second step ProteinA material binds the mouse-anti-IgY antibody and precipitates. This protocol is for the first solution using the one step anti-IgY-antibody slurry.

In this protocol a strong Lysis Buffer is utilized, which is suitable for most types of experiments. It is essential to optimize the concentration of detergents and other components based on the specific proteins and interactions of the study. For certain applications, such as native protein analysis an alternative buffer with milder conditions should be considered.

Material

Cells or Tissue lysate		
Antibodies	Primary Antibody Isotype Control: Normal Chicken IgY	www.davids-science.de www.davids-science.de/NormalChickenIgY
IgY beads	*IgY Bead Slurry:* *https://davids-science.de/p/anti-igy-beads-fuer-immunopraezipitation-ip*	*Alternatively:* https://davids-science.de/p/maus-anti-huhn-unkonjugiert-1-ml
Lysis Buffer	50 mM Tris-HCl pH 6.8 2 % SDS	*Alternative native lysis buffer:* 50 mM TrisHCl 150 mM NaCl 1 mM EDTA 1 mM EGTA 0.2 mM Na_3VO_4 1% Triton X-100

		Add 1 mM PMSF prior to use
Binding Buffer	200 µl Lysis Buffer (1:20) 3800 µl TBS pH 7.5	
Elution Buffer	2% SDS 10 % Glycerol 0.03% Bromphenolblue 0.125 M Tris-HCl pH 6.8 Add 6 µl β-Mercaptoethanol / 100 µl elution buffer freshly before use	Alternatively: In many cases a standard loading buffer for SDS-gels can be used.

- 2 - Method: Preparation

The preparation steps are not necessary. In many cases you can proceed directly with the immunoprecipitation.

Cell Preparation

In this step you prepare your protein solution including the protein of interest that binds the IP antibody. This step is only needed, when you do not have the protein solution of interest.

- Prepare the cell suspension, expressing the protein of interest
  For adherent cells, gently scrape the cells using a cell scraper
- Centrifuge the collected cells at 500 x g and 4°C for 5 minutes
- Discard the supernatant and add 100 µl ice-cold Lysis Buffer for each $1x10^6$ cells
  This should lead to 5 – 10 µg total protein/µl in undiluted samples
- Incubate the cell lysate at 4°C for 45 minutes with periodic inverting of the tube
  When the denaturative lysis buffer is used: Pre heat the lysate buffer to 95°C and incubate the lysing samples for 5 min at 95°C
- Centrifuge the lysate at 12 000 x g and 4°C for 15 minutes
- Collect the supernatant containing the soluble proteins and determine the protein concentration

Wash IgY Beads

In this step you prepare the IgY slurry for the following steps.

- Pipet 40 µl of IgY bead slurry (50%) and 400 µl binding buffer
- Centrifuge at 300 x g for 1 min at 4°C
- Resuspend beads in 40 µl binding buffer

Pre-Clearing

Pre-Clearing is used to minimize the background. Unspecific proteins will bind to the beads. This step is not necessary, but can improve the results.

- Use lysate that corresponds to 50 – 100 µg total protein (in this example 20 µl) and dilute it with TBS 1:20.
  For example: 20 µl Lysate + 380 µl TBS pH 7.5
- Add 40 µl washed IgY bead slurry from previous step to the proteins
- Incubate the sample at 4°C for 1 h without agitation
- Centrifuge at 300 x g at 4°C for 1 minute
- Collect the supernatant containing the pre-cleared lysate and discard pellet with unspecific protein

- 3 - Method: Immunoprecipitation

Antibody Incubation

In this step the IP antibody binds the protein of interest in the lysate

- Add 1 - 20 µl protein specific antibody to 380 µl pre-cleared supernatant
  2 – *10 µg specific antibody is recommended for 500 µg lysate*
- Incubate with slow agitation at 4°C over night

Immunoprecipitation with IgY Beads

The IgY beads are prepared and added to the lysate/antibody mixture. The mouse anti-IgY Beads bind the IP antibody from chicken (IgY). The beads will automatically precipitate during this step.

- Prepare a fresh pre-chilled reaction tube
- Add 40 µl (50% bead slurry) IgY Beads
- Wash bead slurry two times with binding buffer
- Add the beads to the pre-cleared sample/antibody mix
- Incubate at 4°C for 1 hour with gentle agitation
- Centrifuge the tube at 300 x g for 1 min and 4°C

SDS-PAGE Sample preparation

Unspecific proteins are removed by washing the pellet. Afterwards the proteins of interest are eluted from the beads with elution buffer. More gentle elution with glycine gradients are possible and may lead to less background.

- Wash the pelleted beads 3 times with binding buffer
- Elute the immunobinding proteins by resuspending the pellet in 50 µl elution buffer
- Vortex the sample and heat at 95°C for 5 min
- Centrifuge at 300 x g for 1 min
- Supernatant can be directly loaded on a SDS-PAGE gel

- 4 - Trouble Shooting

Reason	Solution
Low Efficiency	Use a highly specific antibody for the target protein. Optimize antibody concentration and incubation time to enhance binding efficiency. Optimize washing conditions to reduce background noise.
Background Signal	Use control samples with a negative control antibody, isotype control. Increase the number of washing steps.
Low Protein Yield	Use an adequate amount of starting material. Consider using a harsher elution buffer, like SDS buffer. Consider adding 0.2% BSA to the glycine elution buffer for stabilizing the eluted proteins.
Bead Aggregation	Reduce the amount of lysate or eluate added to the beads. Optimize the washing conditions to remove excess salt or detergents.
Nonspecific Binding	Consider using sera from the same species and isotype instead of Normal chicken IgY as blocking agent during the pre-clearing step.