

Worksheet IP1

Immunoprecipitation Protocol

Note:

This protocol provides a general method suitable for the immunoprecipitation of radio-labelled antigens. Please note that the choice of radio-labelling procedure may be important in the design of an immunoprecipitation experiment. For the labelling of cell surface antigens lactoperoxidase catalysed iodination with ¹²⁵I is recommended, whilst for intracellular antigens in cultured cells ³⁵S - methionine incorporation would be more suitable (see Practical Immunology, Hudson, C. and Hay, F.C. Blackwell Scientific Publications).

We recommend that suitable controls be used throughout. Appropriate safety precautions should be taken when working with any radioactive isotopes.

1. If using radio-labelled cells lyse 2×10^7 cells in 0.5 ml of cold solubilizing solution. Incubate for 20 minutes on ice.
2. Centrifuge at 1000 x g for 10 minutes to remove insoluble debris.
3. Add lysate to 200 µl of Protein G-Sepharose beads, and incubate overnight at 4°C with tumbling to remove any material that may bind non-specifically to the beads.
4. Simultaneously add 200 µl of the monoclonal antibody of interest (at approximately 10-50 µg/ml) to 100 µl of Protein-G Sepharose beads, and incubate with tumbling overnight at 4°C.
5. Remove beads from cell lysate by centrifugation (11,000 x g. 5 minutes).
6. Wash monoclonal antibody loaded beads twice in phosphate buffered saline, and remove supernatant following centrifugation.
7. Add cell lysate to monoclonal antibody loaded beads, and incubate at 4°C for at least 90 minutes with tumbling.
8. Wash beads 3 times with cold solubilizing solution, harvesting each time by centrifugation.
9. Analyse immunoprecipitate by resuspending beads in SDS-PAGE sample buffer, and running on a suitable SDS-PAGE gel. The gel should be dried and analysed by autoradiography after a suitable exposure period.

Solubilizing solution

	mg/100ml PBS
EDTA	37.0
EGTA	38.0
Iodoacetamide	93.0
Phenylmethylsulfonylfluoride	17.4
Soybean trypsin inhibitor	2.0
Aprotinin (20KIU/ml)	100 µl

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