

A guide to protein detection

Chromatin Immunoprecipitation (ChIP)

Purpose: Investigate the interaction between DNA-binding proteins and DNA in the cell, correlating the localization of proteins or their modifications to regions of the genome; allows study of nuclear processes such as gene regulation as well as pathological conditions

Procedure: Using formaldehyde as reversible cross-linking reagent, chromatin is isolated using antibodies to determine whether the protein or modified protein of interest binds to a specific DNA sequence; real-time PCR is often used to analyze the DNA fragments

Advantages: Genome-wide characterization and high throughput possible; ChIP variations: native ChIP, ChIP-on-chip, ChIP-sequencing, and microChIP

Disadvantages: Numerous steps to make a mistake, extensive optimization, controls are essential to interpret data, highly specific antibody necessary, requires known target sequence, antigen of interest must be accessible in chromatin context

1. Cross-link DNA and proteins (X-ChIP only)

2. Fragmentation of chromatin by micrococcal nuclease (N-ChIP) or sonication (X-ChIP) to generate chromatin fragments

3. Immunoprecipitation of chromatin fragments where target is present

4. Analysis of the immunoprecipitated fraction to determine the abundance of a target DNA sequence (or sequences) relative to input chromatin

DNA analysis

Flow Cytometry

Purpose: Analyze expression of cell surface and intracellular molecules, characterize and define different cell types in heterogeneous cell populations, assess purity of isolated subpopulations, and analyze cell size and volume

Procedure: Cell surface proteins, intracellular proteins or particles are labeled with fluorescently conjugated antibodies, suspended in a stream of fluid, and passed through an electronic detection apparatus measuring scattering and fluorescence properties

Advantages: Allows for simultaneous multi parameter analysis of surface molecules on single cells, more advanced cytometers can also sort the cells based on different parameters

Disadvantages: Complex, expensive, multiple controls needed for multicolor analysis, sorting can be slow and low throughput

Sheath fluid

Sample (stained cells in suspension)

Nozzle

Hydrodynamic Focusing
Cells pass through in 'single file'

Fluorescence emitted from stained cells detected

Forward and side scattered light from all cells detected

Laser light source

Immunoprecipitation (IP)

Purpose: Precipitate and thereby enrich or purify a desired protein out of solution, study protein-protein interactions

Procedure: Antibody for the protein of interest is incubated with cell extract enabling binding of antibody to the protein in solution; antibody-antigen complex will then be pulled from solution using protein A/G-coupled agarose beads, analysis by WB or mass spectrometry

Advantages: Allows for purification or enrichment of protein of interest, multiple variations including precipitation of protein complexes (Co-IP), chromatin immunoprecipitation (ChIP), and RNA immunoprecipitation (RIP)

Disadvantages: IgG remains in sample – if protein of interest has MW of 25 or 50 kDa it will overlap with bands of the IgG's heavy and light chain, validity of protein-protein interaction needs to be confirmed by other methods

Key

- Protein of interest
- Other proteins
- Antibody
- Protein A and G coupled beads

Immunoprecipitate

RNA Immunoprecipitation (RIP) / Nucleotide UV Cross-linking and IP (CLIP)

Purpose: Identify specific RNA molecules associated with specific nuclear or cytoplasmic binding proteins and mapping RNA-protein interactions.

Procedure:
RIP: Precipitation of specific RNA-binding protein and the associated RNA (mRNA, non-coding RNA, viral RNA), followed by detection using real-time PCR, microarrays or sequencing
CLIP: Use of UV cross-linking to irreversibly bind RNA to protein followed by precipitation of specific RNA-binding protein and the associated RNA (mRNA, non-coding RNA, viral RNA); detection by sequencing

Advantages:
CLIP: short antibody incubation time, allows high throughput (HITS-CLIP), other CLIP variations: CLIP-sequencing, PAR-CLIP, iCLIP

Disadvantages:
RIP: Difficult to determine exact binding site (not specific enough), interactions are transient, high background, extensive optimization, controls are essential
CLIP: low cross-link efficiency, expensive, extensive optimization, controls are essential, effective reverse transcription over the cross-link site is often necessary

Key

- Protein of interest
- Other proteins
- RNAs
- Antibody
- Magnet
- Magnetic Bead

Control

Treated

Lyse in Polysome Lysis Buffer

Immunoprecipitate with antibody to protein of interest with protein A/G magnetic beads

Immobilize magnetic bead bound complexes with magnet and wash off unbound material

Extract RNAs

Detect by qRT-PCR, RNase Protection, Microarray, Sequencing

Immunohistochemistry (IHC)

Purpose: Demonstrate the presence and location of proteins in tissue sections

Procedure: Tissue samples (paraffin or frozen) are fixed, blocked, washed, permeabilized (if sections are very thick), incubated with primary antibody, washed, incubated with conjugated secondary, and detected enzymatically or by fluorescence

Advantages: Provides "big picture"; enables the observation of processes in the context of intact tissue, very useful in assessing progression of disease processes such as cancer

Disadvantages: Less sensitive quantitatively than immunoassays such as western blotting or ELISA

Immunohistochemical staining

IHC-P

Deparaffinization and dehydration
Xylene, Xylene 1:1 100% ethanol, 100% ethanol down to 50% ethanol

Antigen retrieval
Heat in citrate buffer pH 6 for 5-20 min
Or
Enzymatic (trypsin, proteinase K)

IHC-Fr and ICC

Fix slides
4% PFA for 10 min
Or Methanol (ice cold) for 10 min
Or Acetone (ice cold) for 10 min

Block with 5% serum or BSA for 30 min to 1 hr

Wash with PBS 0.2% Tween 20, 4°C for 5 minutes

Permeabilize the cells if detecting an intracellular target
0.2% Triton for 10 minutes (not necessary if fixed in acetone or methanol)

Incubate with primary antibody
30 min to 2 hr RT or overnight 4°C

Wash with PBS 0.2% Tween 20, 4°C for 5 minutes

Incubate with conjugated secondary antibody
30 min to 2 hr RT

Enzymatic Detection

Substrate

Colored product

Key

- Cell
- Antigen
- Primary antibody
- Conjugated Secondary antibody

Immunocytochemistry (ICC)

Purpose: Demonstrate the presence and location of proteins in cultured cell samples

Procedure: Cell samples are adhered, fixed, blocked, washed, permeabilized (if intracellular target), incubated with primary antibody, washed, incubated with conjugated secondary, and detected enzymatically or by fluorescence

Advantages: Very useful in determining if cells express a certain protein, provide sub-cellular localization of proteins, and determine co-localization of two or more proteins

Disadvantages: Less sensitive quantitatively than immunoassays such as western blotting or ELISA

Western blot (WB)

Purpose: Detect the presence and approximate size of proteins in a sample

Procedure: Identifies with specific antibodies proteins that have been separated from one another according to their size by gel electrophoresis after transfer to a membrane

Advantages: Highly sensitive and can detect very small (nano to pico molar) amounts of protein

Disadvantages: Numerous steps to make a mistake, background can result from cross reactivity of antibodies, difficult to transfer very large proteins or very hydrophobic membrane proteins

+ve

-ve

Current

Sponge

Filter Paper

Membrane

Gel

Filter Paper

Sponge

100 V - 60 - 120 min 4°C
Optimize time and voltage
Follow manufacturers instructions

Negatively charged proteins move up towards the positive cathode and onto the membrane.

Enzyme-linked immunosorbent assay (ELISA)

Purpose: Detect the presence of antigen in a sample, determine immediate relative concentrations, and absolute concentration by use of standard curve

Procedure: Enzyme linked immunosorbent assay; detects presence of antigen in sample by affixing antigen to 96-well plate, incubating with primary antibody, and washing with conjugated secondary for colorimetric detection

Advantages: Quick, large amount of samples, relatively easy and safe, multiple variations available

Disadvantages: Direct ELISA: minimal signal amplification Indirect ELISA: cross reactivity is possible with secondary antibody

Direct Assay

Indirect Assay

Capture Assay "Sandwich"

AG

E

Substrate

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