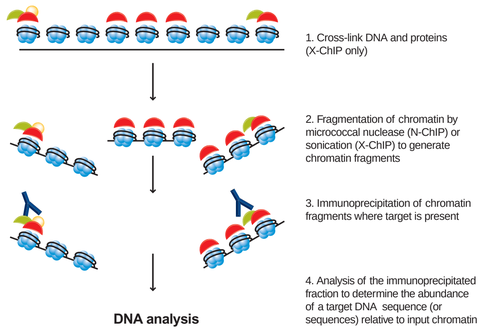
A guide to protein detection

abcam®
discover more

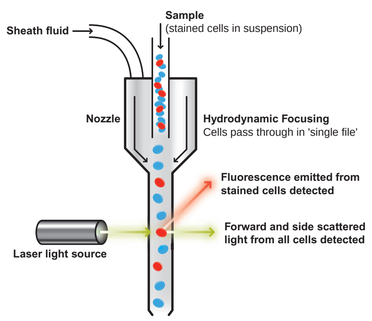
Chromatin Immunoprecipitation (ChIP)

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



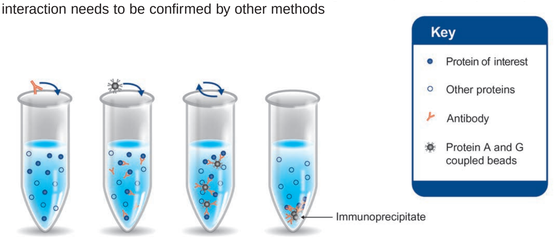
Flow Cytometry

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



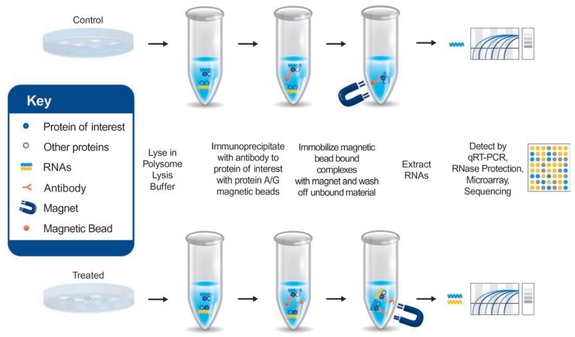
Immunoprecipitation (IP)

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



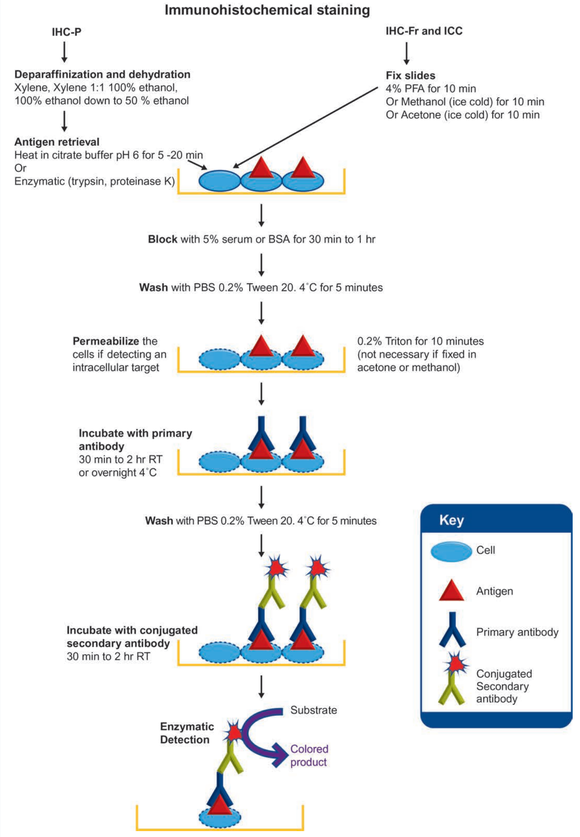
RNA Immunoprecipitation (RIP) / Nucleotide UV Cross-linking and IP (CLIP)

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



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

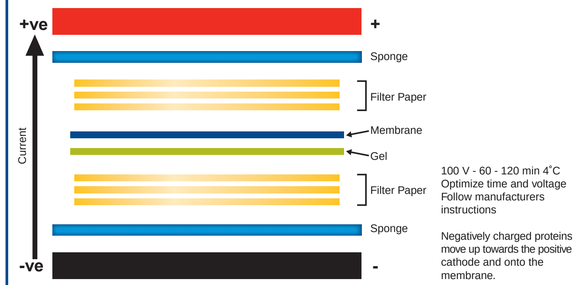


Immunocytochemistry (ICC)

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

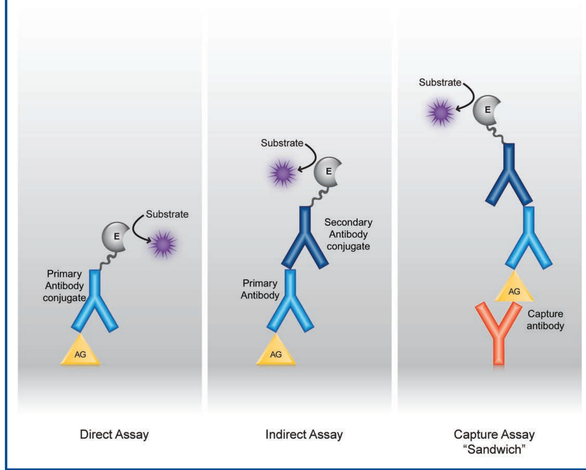
Western blot (WB)

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



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



v001_11_AKE