# A guide to protein detection

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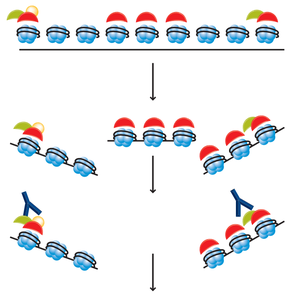
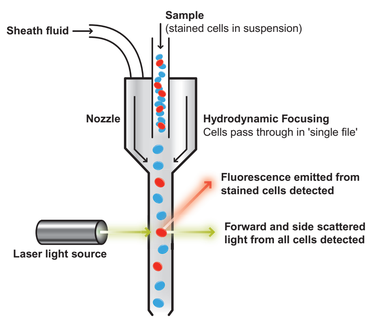
## Chromatin Immunoprecipitation (ChIP)

**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



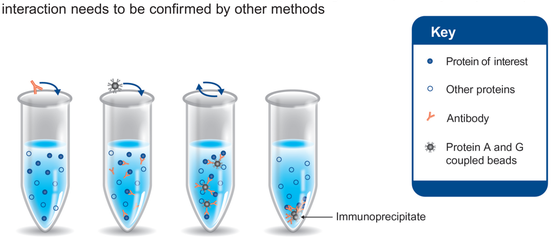

## Flow Cytometry

**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



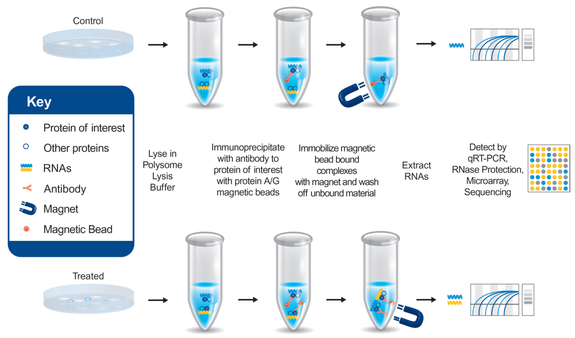

## Immunoprecipitation (IP)

**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



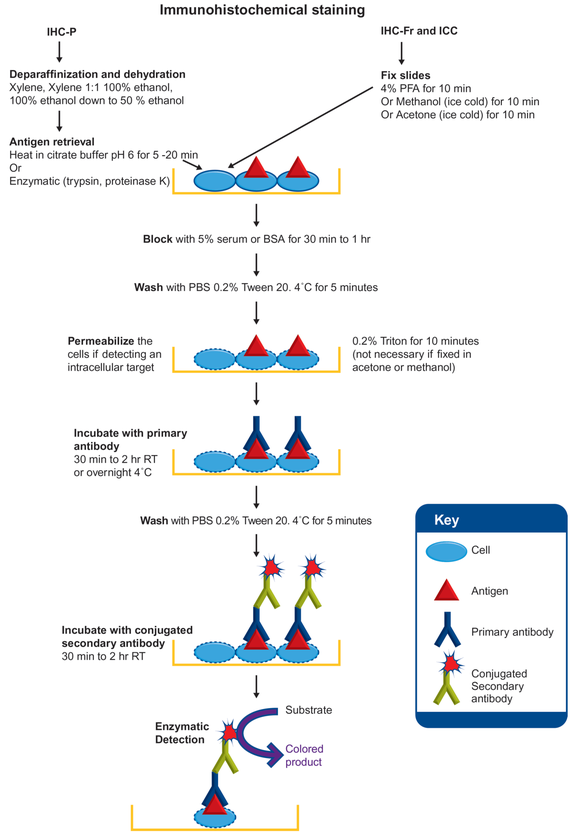

## RNA Immunoprecipitation (RIP) / Nucleotide UV Cross-linking and IP (CLIP)

**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

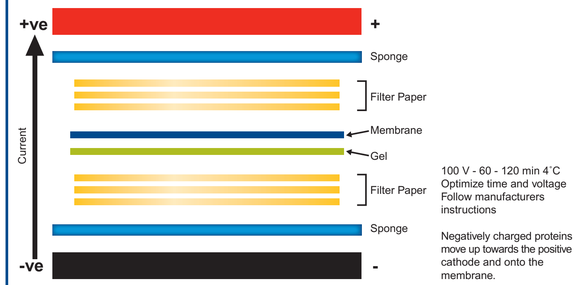



## Immunocytochemistry (ICC)

**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

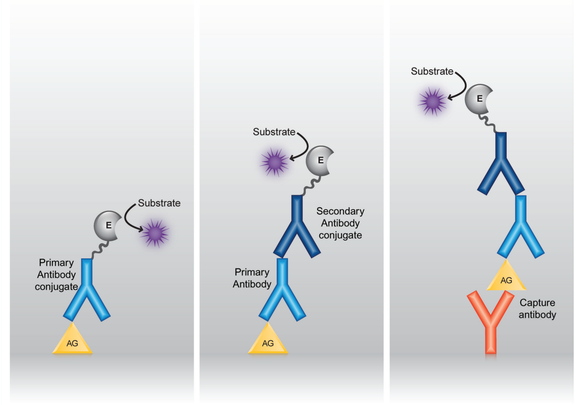
## Western blot (WB)

**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

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