

Immunodetection Fundamentals

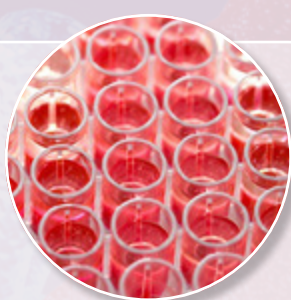
Immunodetection techniques are essential for detecting and quantifying specific proteins or antigens in various samples. Antibody-based approaches like ELISA, western blotting, immunohistochemistry, immunocytochemistry, and immunoprecipitation allow researchers to analyze protein expression levels, localization, interactions, and modifications to improve our understanding of cellular processes, diagnose diseases, develop therapeutics, and advance scientific knowledge.

This infographic provides an overview of the five most commonly used immunodetection techniques, as well as tips to help you determine which method is best for a variety of experimental scenarios and resolve any problems that come up.

Commonly Used Methods

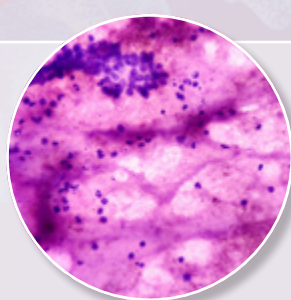
Enzyme-linked immunosorbent assay:

ELISAs use an enzyme-linked antibody to detect the presence of a specific protein or antigen in a sample. The enzyme catalyzes a colorimetric or fluorescent reaction, producing a detectable signal.



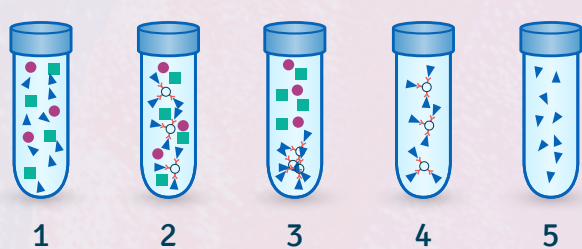
Immunoprecipitation

IP is a form of affinity purification in which targets compounds are purified, or “precipitated” out of a complex solution via specific antibody binding. Once the target has bound to the antibody, there are two ways to collect the antibody-target complex. With pre-immobilized antibodies, the immunoglobulin is covalently linked to a solid support, such as agarose resin or magnetic particles. Alternatively, antibodies could be initially uncoupled, but collected later via immobilized antibody-binding proteins, such as protein A or protein G.



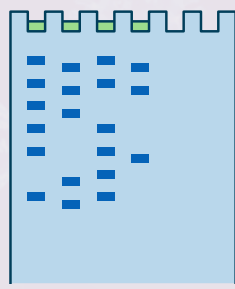
Immunohistochemistry (IHC) and Immunocytochemistry (ICC)

Antibodies are used to detect proteins in tissue sections (IHC) or cells (ICC). Common detection methods include enzyme-based chromogenic detection, fluorescent detection, avidin-biotin complex and polymer-based detection.



Western blotting:

Proteins are separated by gel electrophoresis, transferred to a membrane, and detected using enzyme or fluorescent-labeled antibodies. Common detection methods include HRP or AP and their substrates.



Method Selection Criteria

Factors to consider when choosing the best immunodetection method for your research needs include sample type, desired sensitivity, multiplexing capabilities, and measurement type.

	Sample type	Sensitivity	Multiplexing capability	Quantitative vs Qualitative
ELISA	Best for quantifying soluble proteins or antigens in biological fluids like serum, plasma, or cell culture supernatants, cell lysates, saliva, tissue lysates, and urine	Offer high sensitivity for detecting low-abundance proteins, with chemiluminescent western blotting being the most sensitive	Allow multiplexed detection of multiple targets simultaneously using different antibodies or probes	Results can be quantitative, qualitative, or semi-quantitative
Western Blotting	Ideal for detecting and analyzing specific proteins from complex mixtures like cell lysates or tissue homogenates	Have lower sensitivity compared to ELISA and western blotting	Can also multiplex by using antibodies labeled with distinct fluorophores	No longer limited to generating qualitative data and can be used to generate quantitative data too
IHC and ICC	Used to localize and visualize target proteins in tissue sections or cultured cells, respectively	Sensitivity depends on the abundance of the target protein and the downstream analysis method	Typically used to isolate a single target protein at a time	Generally semi-quantitative or qualitative methods for assessing protein localization and expression levels
IP	Typically intended for the small-scale collection of antigens from lysates or fluids for downstream analysis, such as assays or blotting			Primarily a quantitative technique for isolating and enriching a protein of interest

Problem Solving Resources

ELISA

When ELISAs go wrong, diagnosing and fixing problems can be overwhelming. But whether you're experiencing high background, weak signal or lack of signal, variability between plates or runs, edge effects/plate drift, or poor reproducibility, these **solutions** can help get your assay back on track.



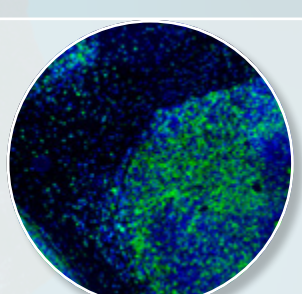
Western blotting

As data incurs more scrutiny than ever before, validated antibodies, appropriate controls, and clear, noise-free, quantifiable blots are critical to the advancement of experiments. Although short-cuts now abound, attention to detail is still paramount to **generating and analyzing the western blot**.



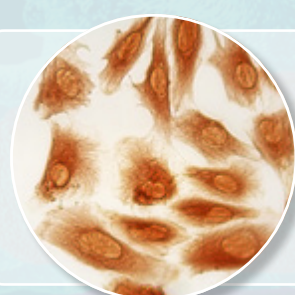
IHC

With IHC, researchers can see where within a tissue a particular protein is localized and can determine its relative expression between different cell types. Although modern IHC benefits from a more streamlined workflow and higher plex compared to early forms of the technique, **care is still required to ensure IHC data is accurate**.



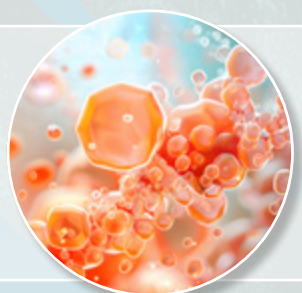
ICC

ICC is a valuable tool for the detection and visualization of proteins in cells using antibodies specifically recognizing the target of interest. Experimental success is **often dependent on the quality of antibodies used as well as the technique**.



IP

Immunoprecipitation with specific antibodies allows the study of native proteins without tags that may alter function. Minimizing non-specific binding and including non-specific antibody controls are **crucial for reliable results** when analyzing protein interactions and functionality in their natural state.



References

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- Antibody Methods and Techniques
- Comparison of Immunostaining Techniques
- How to Hone Your Immunodetection Skills
- How to Improve Western Blot Reproducibility
- How to Select the Best Western Blot Detection Method
- Immunoprecipitation by The Human Protein Atlas
- Infographic: Five Factors to Consider for ELISAs
- Infographic: Immunoassay Troubleshooting Tips
- Infographic: Ten Tips for Developing a Successful Sandwich ELISA
- Infographic: 5 Common ELISA Problems...and How to Fix Them
- Recent Developments in Multiplexing Techniques for Immunohistochemistry
- The Ultimate Quick Tips and Tricks Guide to Western Blots
- Tips for Successful IHC Staining
- Western Blot: Technique, Theory, and Trouble Shooting