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Antibody methods and techniques

An overview of application methods and techniques for use with your antibody.

Enzyme-linked immunosorbent assay (ELISA)

ELISA assays are based upon the principle of antibody/antigen binding. They enable quantification and characterization of specific analytes and/or molecular interactions. Antibodies against the target of interest are conjugated to a reporter enzyme. Upon addition of its substrate, the enzyme catalyzes the production of a colorimetric molecule. The extent of this reaction is measured using a spectrophotometer and is representative of antigen concentration within a sample. The most appropriate ELISA format for each experiment will depend on many factors, including desired sensitivity, specificity and assay time. For the same cost as a standard ELISA, our SimpleStep ELISA™ kits can halve assay time, without compromising sensitivity or reliability.

Western blot (WB)

Western blots are often used to determine relative protein levels between samples. They also establish the molecular weight of the target, which may provide insight into its post-translational processing. Proteins from tissue/cell lysates are separated by gel electrophoresis according to their molecular weight. Following separation samples are transferred to a membrane, where antibodies are applied to probe the protein of interest.

Immunohistochemistry (IHC)

Immunohistochemistry studies elucidate tissue-specific and subcellular localization of an antigen within a sample. Although less quantitive than western blot or ELISA, they offer the advantage of characterizing protein expression in the context of intact tissue. Immunohistochemical staining is accomplished using antibodies that recognize a target protein and antibody-antigen complexes are visualized via chromogenic, radioactive or fluorescent substrates; these can be introduced to the reaction as direct primary conjugates or via a secondary antibody. There are a variety of techniques for sample preparation and visualization, and the method used should be tailored to the type of specimen under investigation and the degree of sensitivity required.

Immunocytochemistry (ICC)

Immunocytochemistry is used to study the subcellular distribution of proteins using fluorescently-labeled antibodies. In contrast with IHC, this technique affords greater spatial resolution as cultured cells lack the complex surrounding environment found in tissue samples. Antibodies raised against a protein of interest are applied to cell culture samples that have been fixed and permeabilized. The binding is visualized by directly conjugated fluorophores or through fluorophore-secondary conjugates. The signal that results can be seen using microscopy. Multiple targets can be studied at once using distinctly-labeled primary antibodies.

Flow cytometry and FACS

Flow cytometry is a means of measuring certain chemical and physical properties of cells. Parameters measured include cell-size and the expression of cell-surface and intracellular markers. Flow cytometry measures the fluorescence emitted by labeled antibodies, bound to individual cells in a mixed population. Fluorescence-activated

cell sorting (FACS) is a more sophisticated system, which quantifies the fluorescent signal and separates the cells from a mixed population that contains preselected characteristics (i.e. fluorescence intensity, size and viability).

Immunoprecipitation (IP)

Immunoprecipitation is a versatile technique that enables isolation and purification of individual and complexed proteins. Antibodies are immobilized on solid-phase substrates (e.g. magnetic/agarose beads), which capture antigens from complex solutions. Chromatin immunoprecipitation (ChIP) is a procedure used to determine whether a given protein binds to a specific DNA sequence in vivo.

Enzyme-linked immunospot (ELISPOT)

ELISPOT is used for the detection of secreted proteins, such as cytokines and growth factors. The technique enables quantification and comparison of immune responses to various stimuli. Cells are grown in 96-well plates with antibody-coated PVDF or nitrocellulose membranes, and the secreted protein of interest binds the antibody. Antibody-antigen interactions are detected using a secondary antibody, and the protein is visualized as a spot color under the parent cell (one spot = 1 cell). Membranes are scanned and analyzed to quantify the number/percentage of cells secreting the protein.