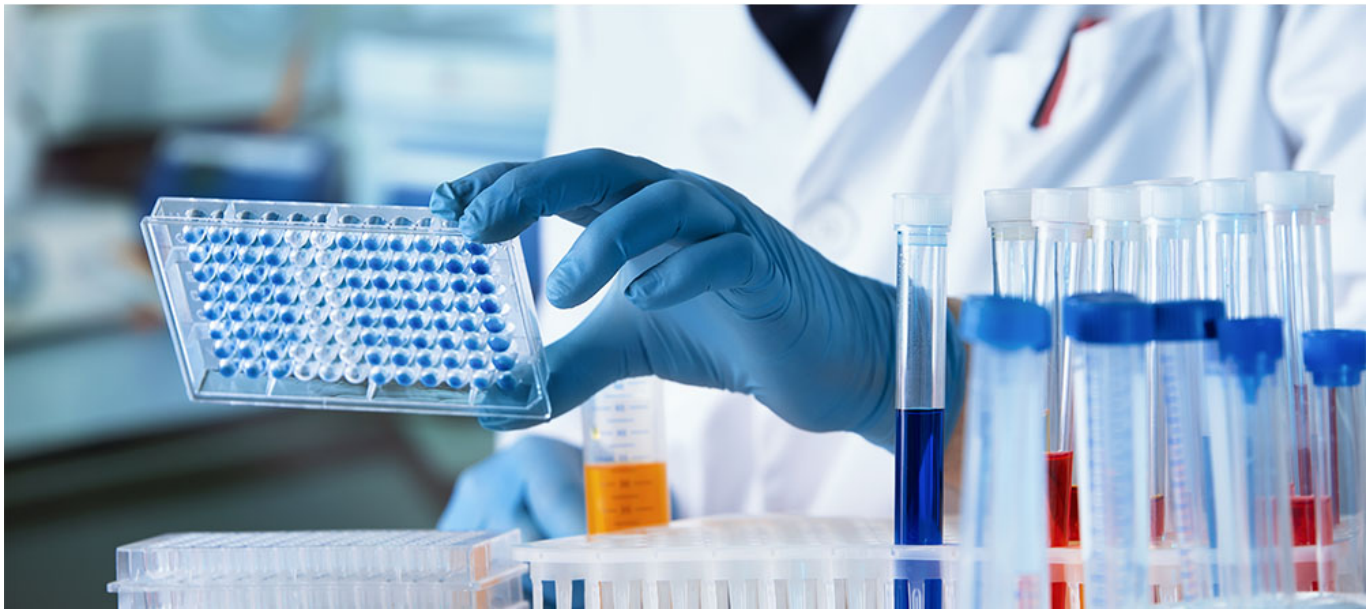
Many different bead-based applications have been developed for detecting and purifying biomolecules. Secondary antibodies have broad utility for these types of techniques due to their wide availability and relatively low cost compared to using labeled primary antibodies. Here, we look at some of the most common bead-based applications, including how they incorporate secondary antibodies. We also comment on what makes a good secondary antibody and suggest features to consider for product selection.



Immunoassay principles

Immunoassays are bioanalytical methods that exploit the specific interaction between an antibody and an antigen to detect targets of interest within sample material. Popular techniques include ELISA, whereby an analyte in solution is either captured by antibodies bound on the surface of a microplate well, or the analytes bind to that surface and are bound by subsequent antibodies; and western blot, which involves gel-based separation of proteins by size, and their subsequent transfer to a membrane for detection by antibodies. In addition, methods such as immunocytochemistry (ICC) and flow cytometry use antibodies to detect biomolecules on or within intact cells, while immunohistochemistry (IHC) uses antibodies to probe solid tissue samples for target antigens.

Bead-based applications

Another class of immunoassays uses beads for analyte detection and/or purification. The following are some of the most widely used bead-based applications:

Lateral flow immunoassays

Lateral flow immunoassays are membrane-based methods used to detect analytes from complex biological solutions such as blood, urine, and environmental samples. Used at the point of care or sampling, they often only offer a qualitative or confirmatory but rapid result. They consist of an absorbent sample pad, to which the sample is applied, adjacent to a conjugate release pad that is loaded with bead-bound analyte-specific antibodies. As the sample passes through the conjugate release pad, the bead-bound antibodies attach to the analyte, which then flows across a nitrocellulose membrane. Here, the analyte-antibody-bead complex is captured by a second analyte-specific antibody that has been immobilized to form a line. Bead accumulation generates a colored signal – red where 40nm gold nanoparticles have been used, or an assortment of colors when using latex microspheres. Another line, consisting of an immobilized secondary antibody with specificity for the bead-bound antibodies, is often included as a control to demonstrate that the lateral flow immunoassay has been performed correctly.

Learn more about Lateral flow tests (LFIA)

Luminex® xMAP® Technology

Luminex xMAP Technology allows for detection of as many as 500 different analytes in the same sample. To achieve this, it uses color-coded beads that are dyed with different ratios of two fluorophores (one red and one infrared) to provide a unique spectral signature. In a typical xMAP assay, spectrally distinct bead sets, each coated with a different analyte-specific antibody, are incubated with the sample. Biotinylated analyte-specific antibodies and a streptavidin-phycoerythrin (PE) conjugate are then added. The amount of bound analyte is measured with a flow cytometry-based Luminex instrument, which uses a red laser to identify each bead set and a green laser to determine the magnitude of the PE-derived signal (directly proportional to the amount of bound analyte). An alternative method uses analyte-coated beads for measuring antibodies in solution. In this scenario, PE-conjugated secondary antibodies or a combination of biotinylated secondary antibodies and a Streptavidin-PE conjugate are often used for detection.

Learn more about Luminex xMAP assays

AlphaScreen™ assays

AlphaScreen (Amplified Luminescent Proximity Homogenous Assay Screen) evolved from a method called a Luminescent Oxygen Channeling Immunoassay (LOCI), which was first described in 1994 by Ullman *et al.* and subsequently commercialized by PerkinElmer1,2. It is based on the use of 'donor' and 'acceptor' beads, respectively, containing a photosensitizer and a chemiluminescent, that are coated with proteins, antibodies, or other biomolecules. When the beads are brought into close proximity by a biological interaction, the donor beads convert ambient oxygen to a singlet state. The singlet oxygen molecules then react with the acceptor beads to cause light emission at 520–620 nm. A popular way of configuring AlphaScreen involves conjugating a secondary antibody to the acceptor bead since this minimizes the use of primary antibodies, which may be expensive or difficult to obtain in sufficient quantities for high-throughput applications.

Magnetic-activated cell sorting (MACS®)

MACS, registered to Miltenyi Biotech, was developed in 1990 as an alternative to fluorescent-activated cell sorting (FACS)3. In the original publication, the authors sequentially stained cells with biotinylated antibodies, fluorochrome-conjugated avidin, and superparamagnetic biotinylated microparticles prior to separation on high-gradient magnetic columns. This method enabled the processing of >109 cells in just 15 minutes, with enrichment rates of more than 100-fold. A main difference between MACS and FACS is that MACS only allows for selecting one marker at a time. However, because MACS is less time-consuming and requires less expensive equipment than FACS, it has utility for many different cell-based applications4. Common approaches to MACS include the use of primary antibody-conjugated microbeads for direct capture and the pairing of a primary antibody with suitable secondary antibody-conjugated microbeads for indirect capture.

Affinity purification

Affinity purification is the process of extracting a protein from solution through its binding to an immobilized ligand. It involves coupling the ligand to a solid support, often a porous gel that can be packed into a column, and allowing the sample to flow freely past the ligand such that the target is captured. After washing to remove any unbound components, the target is eluted with a suitable buffer. Beaded agarose is the most widely used matrix for affinity purification due to its large pore size, low non-specific binding, and stability over a broad pH range, as well as its low cost. Secondary antibodies can be conjugated to beaded agarose for purifying primary antibodies from serum, ascites fluid or cell culture supernatant.

Immunoprecipitation (IP)

Immunoprecipitation is a small-scale form of affinity purification that is widely used for protein enrichment or the study of protein complexes (co-immunoprecipitation/co-IP). During a typical IP reaction, an analyte-specific antibody is immobilized on agarose or magnetic beads before being mixed with the sample. The target of interest, along with any binding partners, is then extracted from solution via centrifugation or magnetic pulldown and eluted for analysis, normally via western blot. A variation of this method, used when the target protein is in low abundance, or the antibody has poor affinity for its antigen, involves mixing the antibody with the sample prior to incubation with the beads. In either setting, the beads must be functionalized to enable antibody binding, usually with Protein A, G or A/G, or a secondary antibody.

Selecting a secondary antibody for bead-based research

When using bead-based applications, choosing the right antibodies is essential for generating accurate and sensitive results. Ensuring that the primary antibody is specific for the target protein is essential. Many manufacturers will provide validation data in the form of identification of purified target protein and absence of detection in knockout cell lines if appropriate. However, it is imperative to establish if the antibody is suitable under your experimental conditions. Once specificity to the target protein has been confirmed, it is then necessary to select a secondary antibody that recognizes the host species of the primary antibody, including the class and sub-class. It may also be useful to determine how the secondary antibody was purified. While Protein A, G, and A/G are frequently used for purification purposes, secondary antibodies that have been purified more specifically against the target antigen have the lowest associated risk of non-specific binding. If you intend to immobilize the secondary antibody onto a bead, you will need to consider the antibody's binding affinity for the biomolecule used for bead functionalization. And, for complete confidence in your chosen product, ensure you purchase your secondary antibody from a trusted supplier. Jackson ImmunoResearch specializes in producing secondary antibodies for life science applications and offers expert technical support to help keep your project on track.

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