

Choosing an antibody

How to select between antibodies based on your application and sample type

Application

Antibody datasheets list the applications we have successfully tested the antibody in. When an antibody is tested in an application and fails, this is also noted on the datasheet. If an application is not listed, it means that we have not tested it and it is unknown how the antibody will perform.

Our antibodies are continuously tested in-house and datasheets are updated with latest application information. For assistance with your application, browse our protocol library.

Nature of sample

The nature of your sample will determine which antibody is most appropriate. Consider the following aspects:

The region of the protein that you wish to detect

Antibodies are generated by immunizing host animals with an immunogenic substance. Immunogens can be full-length proteins, protein fragments, peptides, whole organisms (for example, bacteria), or cells. The immunogen is generally described on the datasheet (in some cases an exact description of the immunogen is not given for proprietary reasons).

Check that the immunogen is identical to or contained within the region of the protein you are trying to detect. For example, if you are trying to detect a cell surface protein on live cells by FACS, choose an antibody that is raised against an extracellular domain of the protein.

Sample processing

Some antibodies require samples to be treated in a specific manner. Many antibodies will only recognize proteins that have been reduced and denatured, because this reveals epitopes that would otherwise be obscured by secondary and tertiary folding of the proteins. On the other hand, some antibodies will only recognize epitopes on proteins in their native, folded state. Our antibodies for western blotting require the samples to be reduced and denatured unless otherwise noted on the datasheet.

For immunohistochemistry, some antibodies are only appropriate for unfixed frozen tissue. Others cannot bind to their targets in formalin-fixed, paraffin-embedded tissues without an antigen retrieval step that reverses the cross-links introduced by formalin fixation. These restrictions on use are noted in the applications section of the datasheets.

Sample species

If possible, choose an antibody that has been raised against the same species your sample is from.

The antibody may react with the same target protein from other species sharing sufficient amino acid sequence homology. If your sample is not from one of the species listed in the datasheet, this means that the species has not been tested and we cannot demonstrate suitability. A prediction of cross-reactivity is made based on sequence similarity.

Choosing the species of primary antibody host

The species the primary antibody is raised in should be different from the species of your sample. This is to avoid cross-reactivity of the secondary anti-immunoglobulin antibody with endogenous immunoglobulins in the sample. For instance, if you are studying a mouse protein, choose a primary antibody that is raised in a species other than mouse. A primary antibody raised in rabbit would be an appropriate choice, followed by an anti-rabbit IgG secondary antibody.

For techniques using samples that do not contain endogenous immunoglobulin (IgG), the choice of host species of the primary antibody is less critical. An example is western blotting of a cell lysate that is not expected to contain IgG. However, tissue lysates and tissue culture supernatants that contain serum will contain immunoglobulins. IgG will appear in western blots of reduced, denatured samples as bands at 50 and 25 kDa corresponding to the heavy and light chains of the IgG molecule.

Choosing a secondary antibody

Secondary antibodies should be against the host species of the primary antibody you are using. For example, if your primary is a mouse monoclonal, you will require an anti-mouse secondary. Check the datasheet of the secondary antibody to ensure it is tested in the application you will be using.

Choosing antibodies for dual staining

Double immunostaining of cell cultures or tissue requires the primary antibodies to be raised in different species, and that the secondary antibodies recognize one of the species exclusively. Choose from our range of secondary anti-Ig antibodies which have been pre-adsorbed against immunoglobulins from other species to remove cross-reactivity. Alternatively, our directly conjugated primary antibodies remove the need for secondary antibodies.

Fluorochrome and chromogen labels

Labels are conjugated to antibodies to visualize presence of the target protein. The choice of label depends on the experimental application.

- Fluorescent labels emit light in the visual range when excited by light of a specific wavelength. There are several available, all with their own excitation and emission characteristics.
- Enzymatic labels horseradish peroxidase (HRP) and alkaline phosphatase (AP) form a colored precipitate when combined with the appropriate substrate.
- Biotinylated antibodies are useful for amplification of signal when followed by an avidin-biotin-enzyme or fluorochrome complex (commonly abbreviated as ABC reagent), or avidin or streptavidin conjugated to an enzyme or fluorochrome.