Secondary Antibody Selection Guide

Our guide will help you choose the most appropriate secondary antibody for your application.

Host species of the primary antibody

The secondary antibody is raised against the host species used to generate the primary antibody, for instance, if you use a primary antibody raised in rabbit, you will need an anti-rabbit secondary antibody raised in a host species other than rabbit (e.g. donkey anti-rabbit secondary).

The experimental procedure

Secondary antibodies tend to come in conjugated forms. For applications such as ELISA or Western blotting, enzyme linked secondaries tend to be the most popular, whereas for flow cytometry or immunofluorescence, there is a preference for secondary antibodies conjugated to fluorescent proteins or dyes such as Alexa Fluor®.

Class/subclass of antibody

The secondary antibody has to be directed against the isotype of the primary antibody.

Polyclonal primary antibodies are generally raised in rabbit, goat, sheep or donkey and are generally IgG isotypes. The secondary antibody therefore, will typically be an anti-IgG H&L (Heavy & Light chains) antibody.

Monoclonal primary antibodies are commonly raised in mouse, rabbit and rat. For example, if the primary monoclonal antibody is a mouse IgG1, you will need an anti-mouse IgG or a less specific F(ab) fragment anti-mouse IgG.

Human immunoglobulin classes, subclasses, types and subtypes

Classes or isotypes: IgG (γ heavy chains), IgM (μ), IgA (α), IgE (ε), IgD (δ)

Subclasses: IgG1 (γ1 heavy chains), IgG2 (γ2), IgG3 (γ3), IgG4 (γ4); IgA1 (α1), IgA2 (α2)

Types: κ light chain, λ light chain

Subtypes: λ1, λ2, λ3, λ4

Other type of reactivities

Polyvalent antibodies react with all classes

Anti-Fc or heavy chain (α, δ, ε, γ, and μ) antibodies react with heavy chain only

Anti-F(ab) or whole molecule antibodies react with heavy and light chains independently of the class

Anti-light chain (κ and λ) antibodies react with all classes since all classes use the same κ and λ light chain

Pre-adsorbed secondary antibodies - ideal for eliminating species reactivity

Pre-adsorbed secondary antibodies are ideal for multi-color experiments when several primary antibodies and their corresponding

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secondary antibodies are used simultaneously. The pre-adsorption process reduces the risk of cross reactivity between the secondary antibody and endogenous immunoglobulins present on cell and tissue samples.

Pre-adsorption (also referred to as cross-adsorption) is an extra step introduced to increase the specificity of an antibody. The mixture of secondary antibodies (containing secondary antibodies against rabbit IgG light chains, sheep IgG light chains and bovine IgG light chains, for example) is passed through a matrix containing immobilized serum proteins from potentially cross reactive species (in this case sheep and bovine light chains).

Only antibodies specific to rabbit IgG light chains will pass through the column whereas, antibodies cross reacting with sheep or bovine IgG light chains will bind and stay adsorbed to the matrix. As a result of the procedure a secondary antibody is generated which specifically recognizes rabbit IgG light chains.

F(ab) or (Fab')2 fragment secondary antibodies

F(ab) and (Fab')2 fragment antibodies eliminate non-specific binding between Fc portions of antibodies and Fc receptors on cells (such as macrophages, dendritic cells, neutrophils, NK cells and B cells), and penetrate tissues more efficiently due to their smaller size.

As fragment antibodies do not have Fc portions, they do not interfere with anti-Fc mediated antibody detection.

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