

SECONDARY ANTIBODIES

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Introduction

In many biological assays, the target antigen is visualized indirectly. That is, the secondary antibody, bound to the primary antibody, produces the signal. Since many secondary antibodies can bind to one primary antibody, the result is signal amplification and increased sensitivity.

The choice of the appropriate secondary antibody is determined by the specifications (i.e. species, subclass, and fragment type) of the primary antibody and the application. Proteintech®* offers a variety of secondary antibodies suitable for Western Blotting, ELISA, cellular imaging, and flow cytometry:

- Enzyme/Biotin conjugated
- Conventional fluorescent conjugated

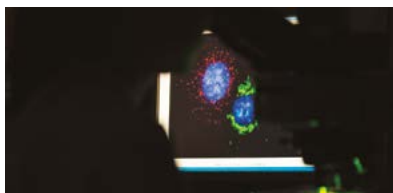
Applications For Secondary Antibodies



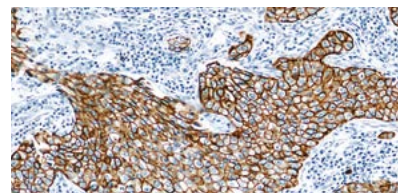
Western Blotting



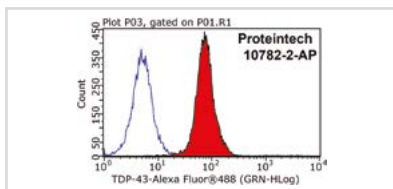
ELISA



Immunofluorescence Staining



Immunohistochemistry



Flow Cytometry

Selecting A Suitable Secondary Antibody For Your Application

Understanding the needs of the primary antibody and assay-dependent requirements are essential for selecting an appropriate secondary antibody.

1. Host Species Of Primary Antibody

The secondary antibody has to be raised against the host species of the primary antibody (e.g., anti-mouse, rabbit, rat).

Ms	Mouse	Gt	Goat	rRb	Rabbit	Sh	Sheep
Rt	Rat	BV	Bovine	Hn	Human	Sw	Swine

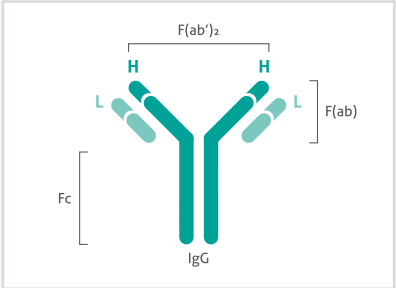
2. Class Of Primary Antibody

In addition to the host specificity, the secondary antibody also has to be specific for the isotype of the primary antibody (e.g., IgG, IgA, IgM).

IgG (H+L), heavy and light chain	IgG3
IgG1	IgG, Fc, receptor binding
IgG2a	IgA
IgG2b	IgM

3. Antibody Fragments

If the primary antibody is not the whole antibody but a fragment, the secondary antibody should also be fragment-specific to reduce noise signal (e.g., F(ab')₂ fragment secondary antibodies penetrate tissue easier in IHC).



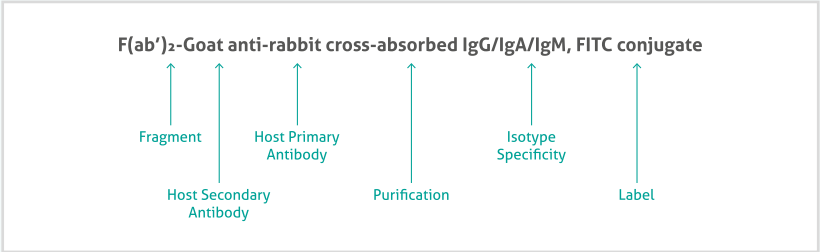
4. Assay Relevance

The intended application determines the type of conjugate (eg., enzyme or fluorescent conjugated).

5. Additional Purification Steps

A common purification technique for secondary antibodies is High Affinity Purification, resulting in reduced non-specific binding. In addition, secondary antibodies can go through an additional purification step to reduce potential cross-reactions with other species. Therefore, the secondary antibody solution is passed over different columns containing sera proteins of different species to filter out the non-specific secondary antibody (e.g., for multi-stainings).

Understanding Common Secondary Antibody Nomenclature



Enzyme Conjugated/ Biotin Secondary Antibodies

Conjugate	Host	Reactivity	Application	Catalog Number
HRP	Goat	Mouse	ELISA, ICC, IHC, WB	SA00001-1
HRP	Goat	Rabbit	ELISA, ICC, IHC, WB	SA00001-2
HRP	Goat	Rat	ELISA, ICC, IHC, WB	SA00001-15
HRP	Goat	Human	ELISA, ICC, IHC, WB	SA00001-17
AP	Goat	Mouse	ELISA, ICC, IHC, WB	SA00002-1
AP	Goat	Rabbit	ELISA, ICC, IHC, WB	SA00002-2
AP	Goat	Rat	ELISA, ICC, IHC, WB	SA00002-9
AP	Goat	Human	ELISA, ICC, IHC, WB	SA00002-8
Biotin	Goat	Mouse	ELISA, FC, ICC, IHC, IF, WB	SA00004-4
Biotin	Rabbit	Goat	ELISA, FC, ICC, IHC, IF, WB	SA00004-1

Conventional Fluorescent Conjugated Secondary Antibodies

Conjugate	Host	Reactivity	Application	Catalog Number
FITC	Goat	Mouse	FC, IF	SA00003-1
FITC	Goat	Rabbit	FC, IF	SA00003-2
FITC	Goat	Rat	FC, IF	SA00003-11
FITC	Goat	Human	FC, IF	SA00003-12
FITC	Rabbit	Goat	FC, IF	SA00003-4
R-PE	Goat	Rabbit	FC, IF	SA00008-2
R-PE	Donkey	Goat	FC, IF	SA00008-3
TRITC	Goat	Mouse	FC, IF	SA00007-1
TRITC	Goat	Rabbit	FC, IF	SA00007-2
TRITC	Goat	Human	FC, IF	SA00007-10
TRITC	Rabbit	Goat	FC, IF	SA00007-4
Cy3	Goat	Mouse	FC, IF	SA00009-1
Cy3	Goat	Rabbit	FC, IF	SA00009-2
Cy3	Rabbit	Goat	FC, IF	SA00009-4



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