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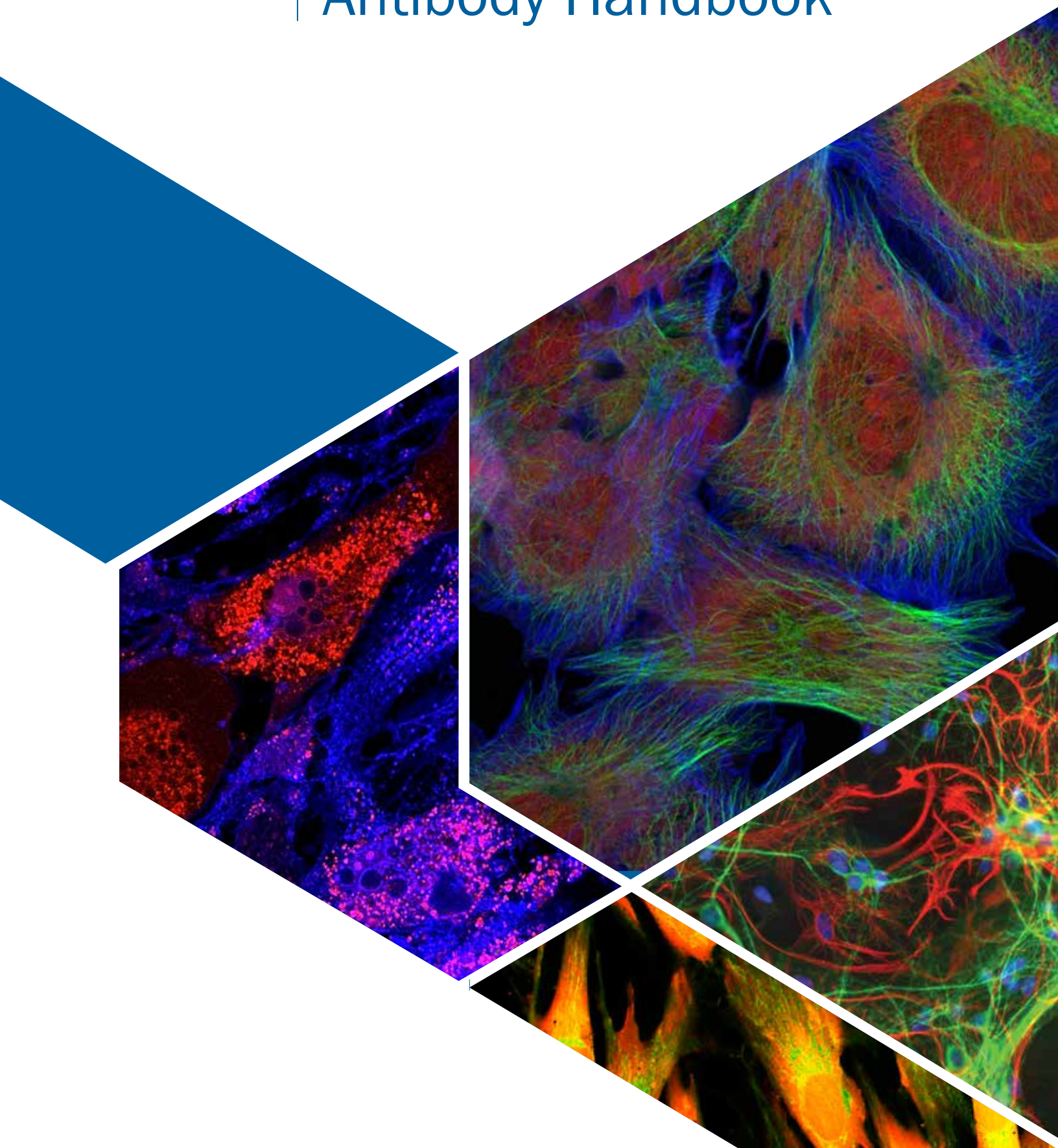
Secondary Antibody Handbook



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INTRODUCTION

A secondary antibody is used to detect protein expression in a cell or tissue sample. In conjunction with an antigen specific primary antibody, a secondary antibody allows highly specific amino acid sequences within a target protein to be detected. The specificity of a secondary antibody for a designated region or regions of a primary antibody allows multiple secondary antibodies to bind to a single primary antibody, amplifying signal and increasing assay sensitivity. Signal amplification and increased assay flexibility due to the diversity of labeled commercially available secondary antibodies are two distinct benefits to consider when designing your experiment.

In this guide, we will provide a succinct introduction to antibody structure and a comprehensive overview of secondary antibody nomenclature. We will also provide pertinent technical tips and important scientific information for you to consider when choosing a secondary antibody for your experiment.

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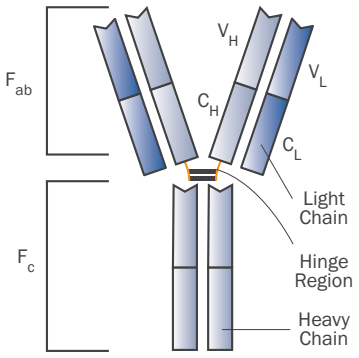
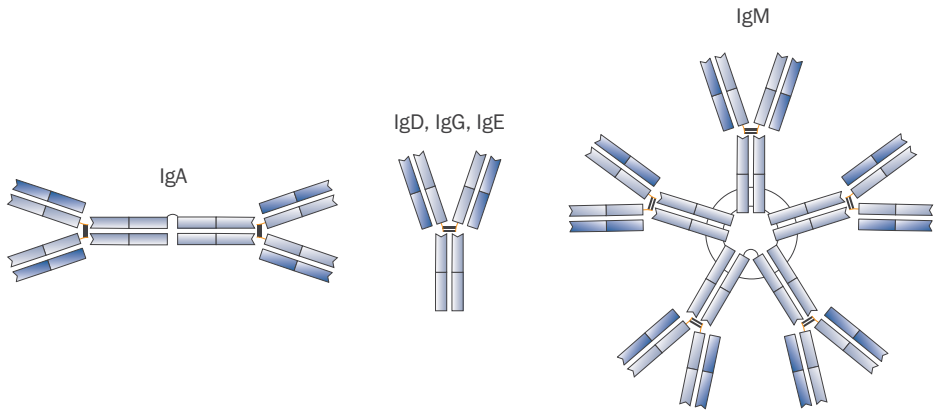
ANTIBODY STRUCTURE

An antibody is a Y-shaped molecule composed of three equal-sized regions. A flexible hinge joins the antibody stalk (Fc) to the arms of the antibody (Fab). The two arms function to bind antigen, while the stalk region determines the antibody's isotype and functional properties. Each arm is composed of one heavy and one light chain. Furthermore, these heavy and light chains are made up of one variable domain (V_L or V_H) and one constant domain (C_L or C_H). The variable regions (V) of the heavy and light chains differ between antibodies and confer antibody specificity for its cognate antigen. The Fc stalk demonstrates little variability, but is important for interactions with effector molecules and cells.

Antibody Structure



Antibody Isotypes



Commonly Used Abbreviations for Secondary Antibodies

Abbreviation	Definition	Description
Ig	Immunoglobulin	Also known as an antibody
F(ab)	Fragment antigen-binding	One constant and one variable domain of the heavy and light chains (one arm of antibody)
F(ab')	Fragment antigen-binding	A F(ab) fragment plus the hinge region
$F(ab')_2$	Fragment antigen-binding	Whole Fab region (two arms of the antibody) with the hinge, no Fc region
Fc	Fragment crystallizable region	Heavy chains forming the antibody stalk and hinge
C_L	Constant domain light chain	
C_H	Constant domain heavy chain	
V_L	Variable domain light chain	Contains antigen binding site, confers antibody specificity
V_H	Variable domain heavy chain	Contains antigen binding site, confers antibody specificity
(H+L)	Heavy + light	Whole immunoglobulin (heavy and light chain)
α	Alpha heavy chain	IgA class
δ	Delta heavy chain	IgD class
ε	Epsilon heavy chain	IgE class
γ	Gamma heavy chain	IgG class
μ	Mu heavy chain	IgM class
κ	Kappa light chain	
λ	Lambda light chain	

SECONDARY ANTIBODY NOMENCLATURE:
Understanding how Secondary Antibodies are Named

Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

Host Species: The species used to raise the secondary antibody.
Format: The structure of the secondary antibody.
Species Reactivity: The species recognized by the secondary antibody.
Target Immunoglobulin: The immunoglobulin class or subclass targeted by the secondary antibody.
Specificity: The region of the target immunoglobulin recognized by the secondary antibody.
Detection Label: The label or conjugate attached to the secondary antibody.

What is a secondary antibody?

A secondary antibody is an antibody directed against an immunoglobulin (antibody) molecule. Under optimal conditions, a secondary antibody specifically binds the species and class (isotype) of the primary antibody in an immunoassay (indirect detection). Note that a secondary antibody does not directly bind to the target protein epitope and does not confer target epitope specificity, which is determined by the primary antibody. A labeled secondary antibody determines the method of detection.

How is a secondary antibody made?

A secondary antibody is produced and harvested from an animal immunized with an antibody from another species. The specifications of the immunizing antibody (e.g. species, subclass, fragment, etc) determine the specificity of the secondary antibody produced. For example, a rabbit anti-rat IgG2b (H+L) secondary antibody is produced by immunizing a rabbit with the heavy and light chains of a rat IgG2b immunoglobulin molecule.

What are the benefits of a secondary antibody?

A secondary antibody increases assay sensitivity due to the ability of multiple secondary antibodies to bind to a single primary antibody (signal amplification). The use of a secondary antibody also affords more flexibility in multiple labeling experiment since commercially available secondary antibodies are offered preconjugated to diverse labels.

Benefits of a Secondary Antibody

Direct Detection: A single conjugated antibody directed against the target of interest.

Indirect Detection: An unconjugated primary antibody is used in conjunction with a conjugated secondary antibody.

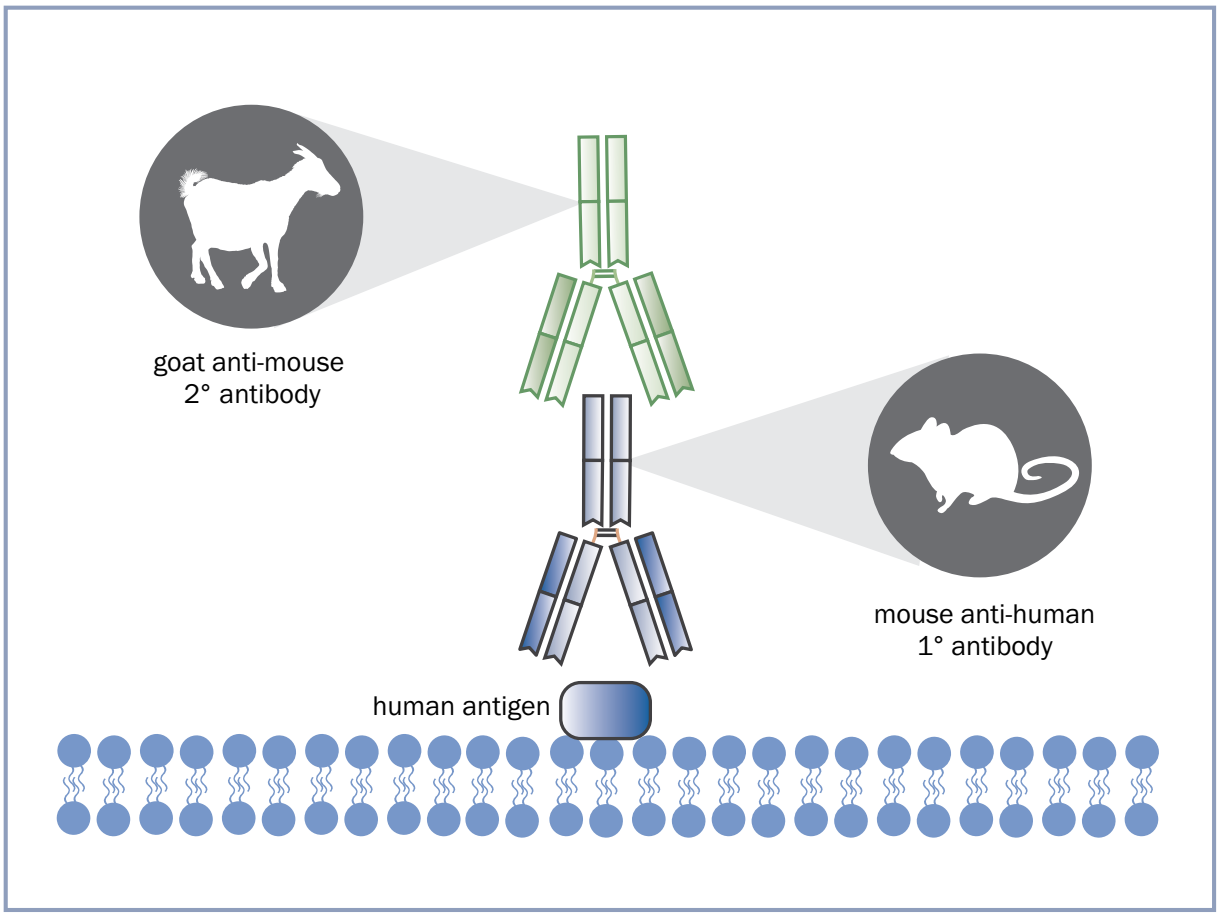
	Direct	Indirect
Sensitivity	Weaker signal since amplification through secondary antibodies is not possible.	Multiple secondary antibodies bind a single primary antibody resulting in signal amplification.
Flexibility	Less flexibility due to offering of commercially available conjugated primary antibodies.	More flexibility due to more combinations of primary antibodies with labeled secondary antibodies.
Time	Shorter protocols since direct detection requires one step labeling.	Longer protocols since indirect detection requires two step labeling.

SECONDARY ANTIBODY HOST SPECIES:
Choosing the Right Host Species

Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

What species secondary antibody should I use?

A secondary antibody should be directed against, but not raised in, the same species as the primary antibody. For example, a mouse primary antibody requires an anti-mouse secondary antibody raised in any species other than mouse (e.g. rabbit, goat, rat, donkey, etc.).



TIPS: When considering host species

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When possible, all secondary antibodies should be derived from the same host species in multiple labeling experiments. However, all primary antibodies should be raised in unique host species. This allows the use of species-specific secondary antibodies directed against one primary antibody which limits cross-reactivity between the secondary antibody and primary antibodies from other species (See page 30).

To limit a secondary antibody from binding a primary antibody raised in a closely related species, use a secondary antibody pre-adsorbed against the related species. For example, an anti-mouse secondary antibody may cross-react with a rat primary antibody in a multiple labeling experiment. Using an anti-mouse secondary antibody adsorbed against rat may reduce cross-reactivity. **Note:** Care should be given when considering secondary antibodies pre-adsorbed against closely-related species (e.g. rat and mouse). Adsorption may eliminate high affinity antibodies and result in weaker signal. See page 24 for more information about pre-adsorbed antibodies.

02

03

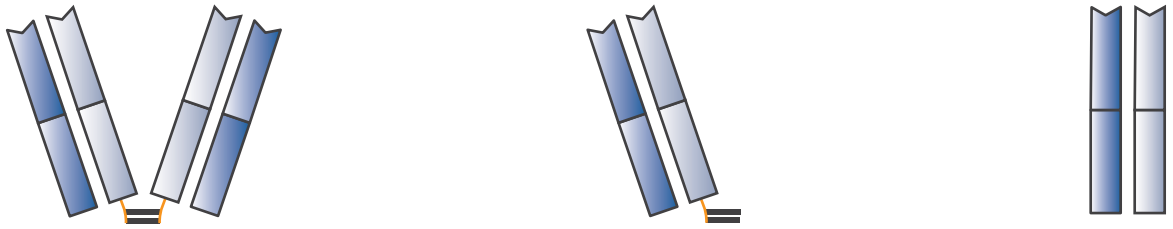
To reduce non-specific staining and reduce background signal, block with serum from the same host species as the secondary antibody.

SECONDARY ANTIBODY FORMAT:
Choosing the Right Format

Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

The format of a secondary antibody refers to the structure of the secondary antibody itself. Whole antibody can be cleaved by pepsin to eliminate the Fc portion of the antibody, producing a $F(ab')_2$ fragment antibody: two arms of the antibody and the hinge region. Further cleavage of the $F(ab')_2$ fragment with β-mercaptoethanol removes one arm of the $F(ab')_2$ fragment, producing a F(ab') fragment: one arm of the antibody and the hinge region. Note that a F(ab') fragment differs structurally from a F(ab) fragment: a F(ab) fragment is formed by papain cleavage of whole immunoglobulin and lacks the hinge region present in a F(ab') fragment.

F(ab) Antibody Fragments



$F(ab')_2$
after pepsin cleavage

F(ab')
after pepsin and β–mercaptoethanol

F(ab)
after papain cleavage

Note: When considering a secondary antibody, it is important not to confuse the format with the specificity. Consider this secondary antibody: Goat $F(ab')_2$ Anti-Human IgG F(ab) Secondary Antibody. The first $F(ab')_2$ designation refers to the secondary antibody format, while the second F(ab) refers to the secondary antibody specificity. In this example, the secondary antibody consists of the $F(ab')_2$ portion (format) and is specific for the F(ab) region of the primary antibody it's targeting (specificity).

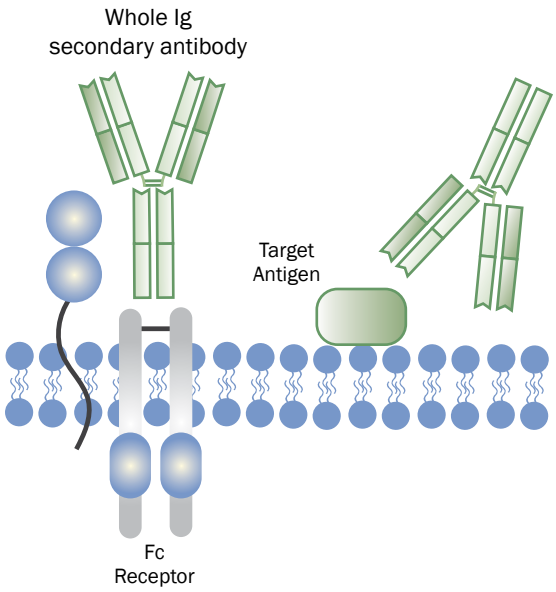
When should I use a Fab fragment secondary antibody?

To Stain Cells with High Fc Receptor Expression

Fc receptors expressed on the cell surface of leukocyte populations (e.g. macrophages, B lymphocytes, natural killer cells, etc.) can bind the antibody's Fc portion, resulting in increased non-specific binding and background signal. A Fab fragment secondary antibody is recommended when staining tissue or cells expressing high amounts of Fc receptor (e.g. lymph node, spleen, peripheral blood, etc.). The lack of the Fc portion of the secondary antibody eliminates secondary antibody binding to Fc receptors expressed in the sample.

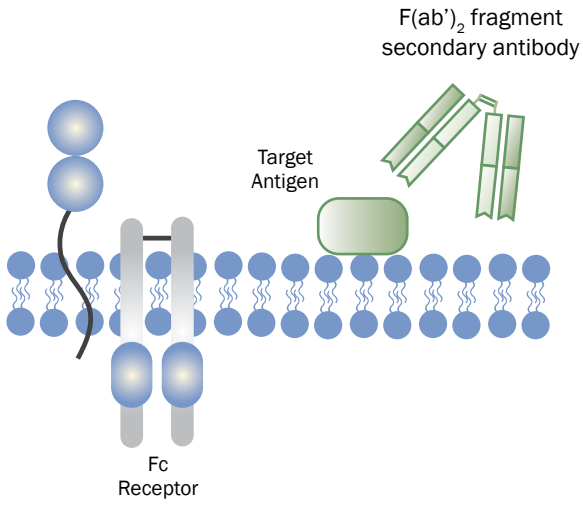
$F(ab')_2$ fragment secondary antibody staining of Fc receptor-expressing cells or tissues

Whole Ig Secondary Antibody

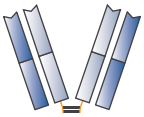
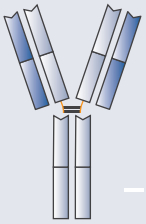


Whole Ig secondary antibody binds Fc receptor in the sample increasing background signal.

$F(ab')_2$ Fragment Secondary Antibody



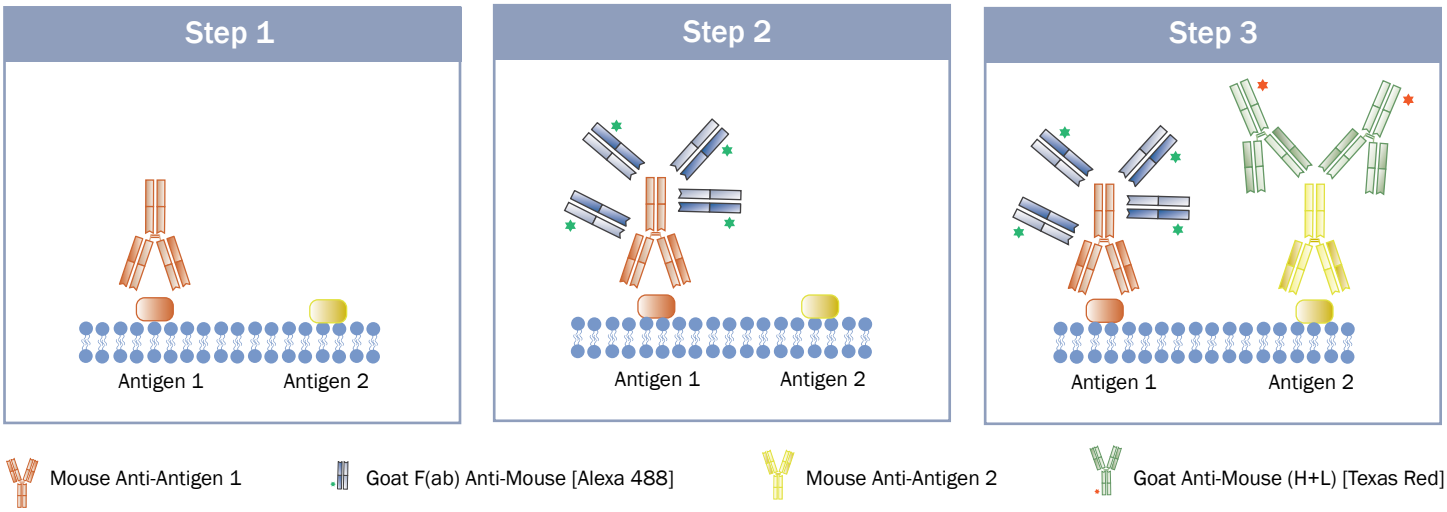
$F(ab')_2$ fragment secondary antibody does not bind Fc receptor in the sample.

Format	Description	Structure	Monovalent or Divalent	Advantages
F(ab)	One arm of the antibody		Monovalent: One antigen binding site	Reduce non-specific binding between Fc receptors and the Fc portion of the antibody. Fragment antibodies penetrate tissue better than whole antibodies due to their smaller size. This may enhance staining in IHC.
F(ab')$_2$	Two arms of the antibody		Divalent: Two antigen binding sites	Reduce non-specific binding between Fc receptors and the Fc portion of the antibody. Fragment antibodies penetrate tissue better than whole antibodies due to their smaller size. This may enhance staining in IHC.
Whole Antibody	Intact antibody		Divalent: Two antigen binding sites	Compatible with most assays. Tend to be less expensive.

To Double Label with Primary Antibodies from the Same Host Species

F(ab) fragment secondary antibodies can be used to double label with two primary antibodies from the same host species. Because each F(ab) fragment secondary antibody contains only one antigen binding site (monovalent), it cannot bind another molecule (i.e. the second primary antibody when double labeling) upon subsequent incubation. $F(ab')_2$ secondary antibodies are divalent (two binding sites) and are not recommended for double labeling experiments. After binding the first primary antibody, the second binding site of a divalent secondary antibody may be left unoccupied. This unoccupied binding site is then capable of binding the second primary antibody, resulting in false positive error.

Double Labeling with F(ab) Fragment Secondary Antibodies



1. Incubate the sample with the first primary antibody (e.g. Mouse Anti-Antigen 1).
2. Incubate with excess of the conjugated F(ab) fragment secondary antibody (e.g. F(ab) fragment Goat Anti-Mouse [Alexa 488]). Wash.
3. Incubate the sample with the second primary antibody (e.g. Mouse Anti-Antigen 2).
4. Incubate with the second conjugated secondary antibody (e.g. Goat Anti-Mouse (H+L) [Texas Red]).

To Block Endogenous Immunoglobulin

Secondary antibody binding to endogenous immunoglobulins can increase background signal. Incubating sample with unconjugated Fab (H+L) antibody after blocking with normal serum will reduce background signal and increase specific staining.

SECONDARY ANTIBODY TARGET IMMUNOGLOBULIN: Choosing the Right Immunoglobulin

Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

What are antibody or immunoglobulin classes (isotypes)?

Slight variation in the Fc portion of the antibody's heavy chain allow antibodies to be divided into five unique classes: IgA, IgD, IgE, IgG, IgM. The heavy chains of the five classes are denoted by their corresponding Greek letter: α, δ, ε, γ, and μ, respectively. Furthermore, the IgG and IgA isotypes can be divided into subclasses due to further heavy chain variation. Unlike an antibody's heavy chains, the light chains, lambda (λ) and kappa (κ), are shared between all classes. However, each antibody only expresses one light chain or the other, never both.

Secondary Antibody Classes and Subclasses

Immunogloublin Classes	IgA	IgD	IgE	IgG	IgM	
Ig Subclasses – Human	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2
Ig Subclasses - Mouse	IgG1	IgG2a	IgG2b	IgG3		
Light Chains	Kappa	Lamba				
Heavy Chains	IgG (gamma)	IgM (mu)	IgA (alpha)	IgD (delta)	IgE (epsilon)	

What immunoglobulin class or subclass should I use for my primary antibody?

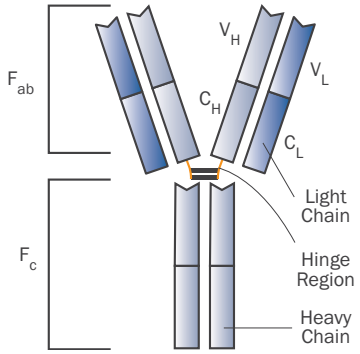
Monoclonal Antibodies: A secondary antibody should be directed against the class or subclass of the primary antibody. For example, a mouse IgM primary antibody requires an anti-mouse IgM secondary antibody. At times, a specific subclass (e.g. human IgG2, IgG1) may be recommended. In this case, a secondary antibody directed against the more general class (i.e. anti-human IgG) can be used for single labeling experiments, since most class-specific secondary antibodies will recognize individual subclasses. However, subclass specific secondary antibodies should be used to differentiate between primary antibodies in multiple labeling experiments when more than one subclass specific primary antibody is used. For example, a multiple staining experiment using IgG2 and IgG1 primary antibodies requires anti-IgG2 and anti-IgG1 secondary antibodies.

Polyclonal Antibodies: When a polyclonal primary antibody is used, then an anti-IgG secondary is recommended since most polyclonal antibodies are IgG class immunoglobulins.

SECONDARY ANTIBODY SPECIFICITY:
Choosing the Right Specificity

Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

Note: As mentioned in the Secondary Antibody Format section, it is important not to confuse the format of the secondary antibody with its specificity. Specificity refers to the region or chain of the primary antibody or immunoglobulin recognized by the secondary antibody.



Anti-IgG (H+L): A secondary antibody with the (H+L) designation targets the heavy and light chains of the IgG molecule (i.e. Fc and Fab regions). These antibodies also react with other classes (e.g. IgE, IgD, etc.) due to shared light chains between classes. The broader epitope recognition of (H+L) secondary antibodies makes them applicable for most immunoassays requiring a secondary antibody.

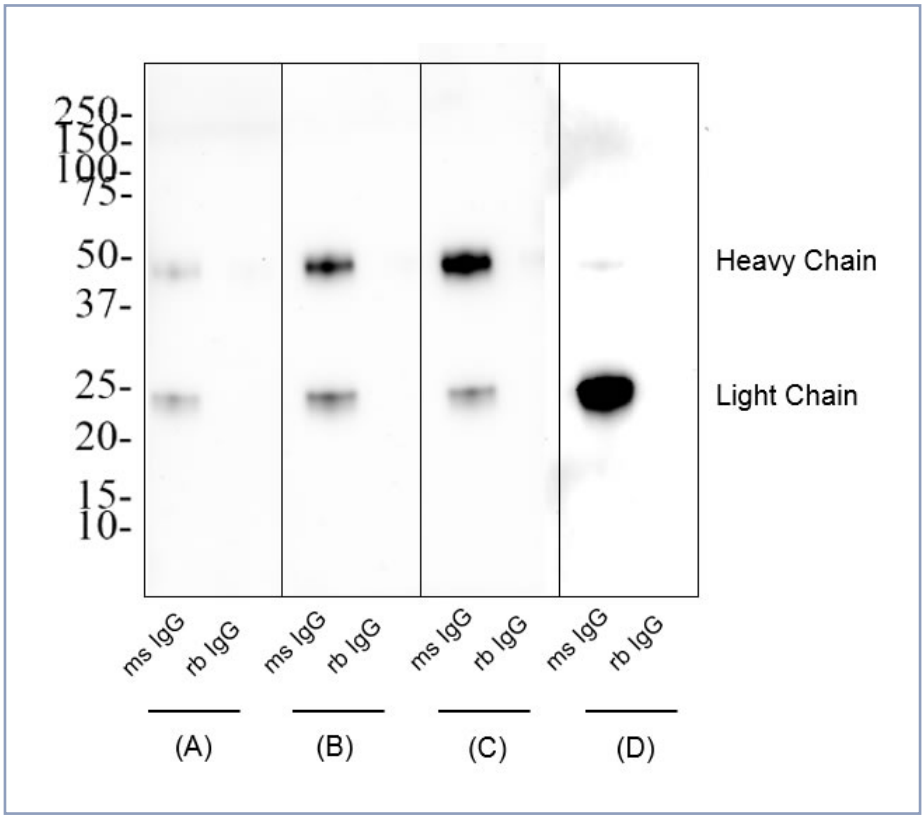
Anti-IgG Fc fragment or heavy chain: Unlike (H+L) secondary antibodies, a secondary antibody targeting the Fc region or heavy chains of the target immunoglobulin is class specific and will not react with other immunoglobulin classes. Secondary antibodies specific for the Fc fragment have been adsorbed against $F(ab')_2$ fragments. A heavy chain specific secondary antibody is recommended to probe expression of a protein larger than 55 kDa or smaller than 45 kDa if western blot analysis is intended post-IP. A secondary antibody specific for the (H+L) region will recognize the reduced and denatured heavy chains (band at 50 kDa) and light chains (25 kDa) of the primary antibody utilized for immunoprecipitation. In addition, Fab fragment anti-Fc secondary antibodies can be used to pre-label primary antibodies before incubation in multiple labeling experiments.

Anti-IgG $F(ab')_2$ fragment: A secondary antibody with the $F(ab')_2$ designation targets the $F(ab')_2$ region of the IgG primary antibody. These antibodies have been adsorbed against Fc fragments, but do react with other classes due to recognition of the shared light chain in the F(ab) region.

Anti-IgG light chain: A light chain specific secondary antibody targets the IgG light chains (kappa or lambda). These antibodies do not react with the primary antibody heavy chains, but do recognize the shared light chains of other immunoglobulin classes. For example, a Goat Anti-Human IgG Kappa Light Chain Secondary Antibody will not only recognize the IgG subclass, but will recognize the light chains of IgA, IgD, IgE, and IgM. A light chain specific secondary antibody is recommended to probe expression of a protein greater than 30 kDa when western blot analysis is run post-IP. A (H+L) specific secondary antibody used in western blot analysis will recognize both the heavy (50 kDa) and light chain (25 kDa) of the reduced and denatured primary antibody used in the immunoprecipitation step. By using the light chain specific secondary antibody, the heavy chain band at 50 kDa is eliminated and proteins greater than 30 kDa can be detected with clarity.

Secondary Antibody Specificities

Type	Specificity	Fc specificity?	F(ab) specificity?	Class Specific?
Anti-IgG (H+L)	Heavy and light chains of primary antibody	Yes	Yes	No, will react with light chains of IgA, IgD, IgE, and IgM.
Anti-IgG Fc Fragment or Heavy Chain Specific	Heavy chains only of primary antibody	Yes	No	Yes, only reacts with IgG
Anti-IgG F(ab) or $F(ab')_2$ Fragment	Heavy and light chains of primary antibodies	No	Yes	No, will react with light chains of IgA, IgD, IgE, and IgM.
Anti-IgG Light chain (kappa, lambda)	Light chains only of primary antibody	No	Yes	No, will react with light chains of IgA, IgD, IgE, and IgM.



Mouse and rabbit immunoglobulins were denatured, separated by SDS-PAGE, and their expression was probed by Western blot analysis with the following secondary antibodies used at 1:5000 dilution:

(A) Gold Standard Competitor, Goat Anti-Mouse IgG (H+L)
(B) Novus Biologicals, Goat Anti-Mouse IgG (H+L)
(C) Novus Biologicals, Goat $F(ab')_2$ Anti-Mouse IgG (H+L)
(D) Novus Biologicals, Goat Anti-Mouse IgG kappa light chain

TIPS: When considering specificity

01

When the subclass of the primary antibody is unknown, a secondary antibody targeting the light chain is recommended. In this case, an anti-IgG (H+L) secondary antibody is most commonly used.

Secondary antibodies targeting the light chain will bind to any class; all classes express the light chains.

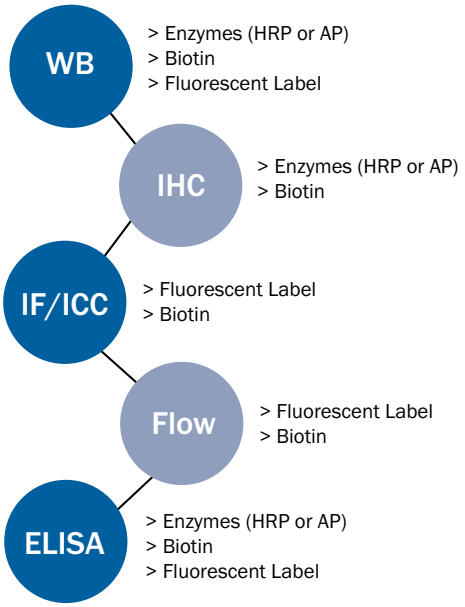
02

SECONDARY ANTIBODIES AND CONJUGATES: How to Determine the Correct Label

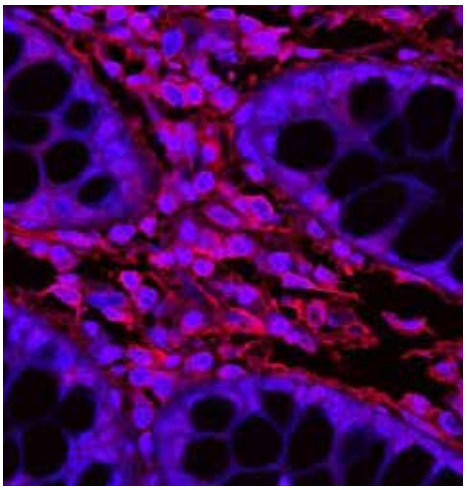
Goat	F(ab)	anti-Rabbit	IgG	(H+L)	Secondary Antibody	[PE]
Host Species	Format	Species Reactivity	Target Ig	Specificity		Label

What label should I choose?

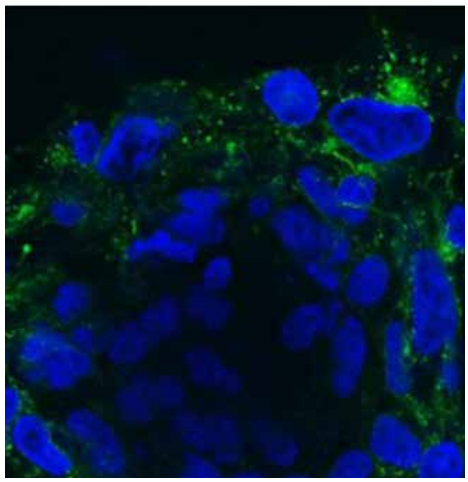
The choice of label is application dependent. Assays requiring fluorescent detection (e.g. flow cytometry, immunocytochemistry, immunofluorescence, etc) require a secondary antibody conjugated to a fluorochrome. The excitation and emission spectra of each fluorochrome should be considered when designing each experiment. Common fluorochromes include FITC, PE, APC, DyLight™ AlexaFluor™, or Atto dyes. Enzymatic detection requires a secondary antibody conjugated to HRP, Alkaline Phosphatase (AP), or biotin. The ability of avidin and streptavidin to bind biotin and form complexe enables signal amplification independent of the host species of the secondary antibody. Because peroxidase is economical and more stable than AP, HRPs more popular for chemiluminscent detection systems. However, the enhanced sensitivity of AP compared to HRP makes AP more common in colorimetric detection assays.



Fluorescent detection using a fluorochrome conjugated secondary antibody:

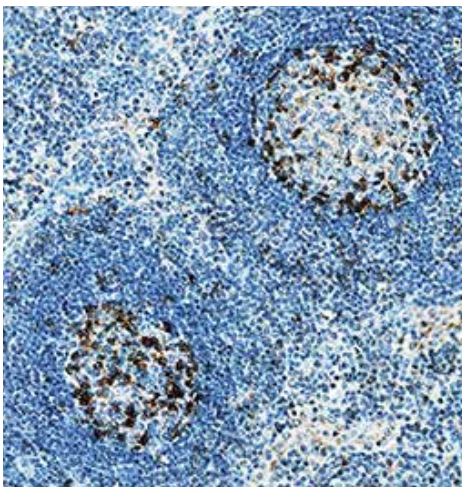


Immunofluorescence: Goat Anti-Mouse IgG Antiboday [Cy5] [NB7602] staining of human colon tissue.

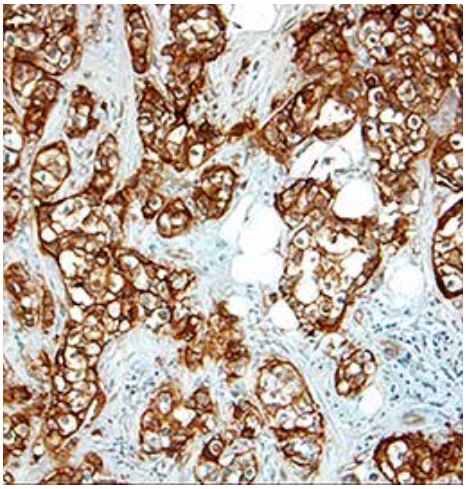


Immunocytochemistry: Goat-Anti-Mouse IgG (H+L) Antibody [Dylight 488] [NBP1-72874] staining of MDA-MB-31.

Chromogenic detection using an HRP conjugated secondary antibody:

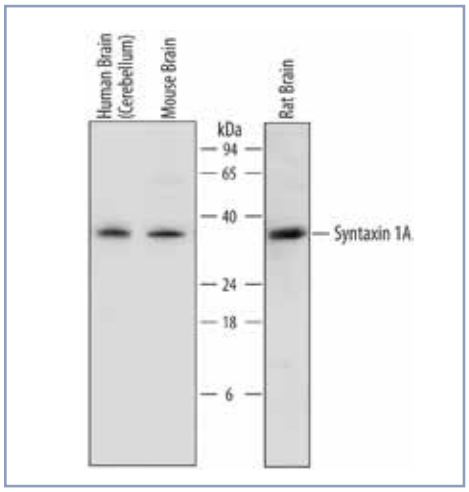


Immunohistochemistry: Anti-Goat HRP Secondary Antibody staining of human lymph node with Goat Anti-Human PD-1 [AF1086].

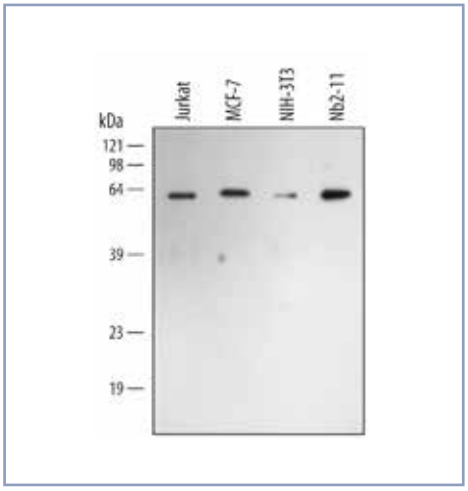


Immunohistochemistry: Anti-Goat HRP Secondary Antibody staining of human breast cancer tissue with Goat Anti-Human Her2 [AF1129].

Chemiluminescent detection using an HRP conjugated secondary antibody:



Western Blot: Rabbit Anti-Goat IgG Secondary Antibody [HRP] [HAF017] staining of brain lysate with Goat Anti-Syntaxin 1A [AF7237].



Western Blot: Goat Anti-Rabbit IgG Antibody [HRP] [HAF008] staining of cell line lysates with Rabbit Anti-Human/Mouse/Rat HSP60 [AF1800].

SECONDARY ANTIBODIES AND PRE-ADSORPTION: How to Limit Cross-Reactivity

What is pre-adsorption?

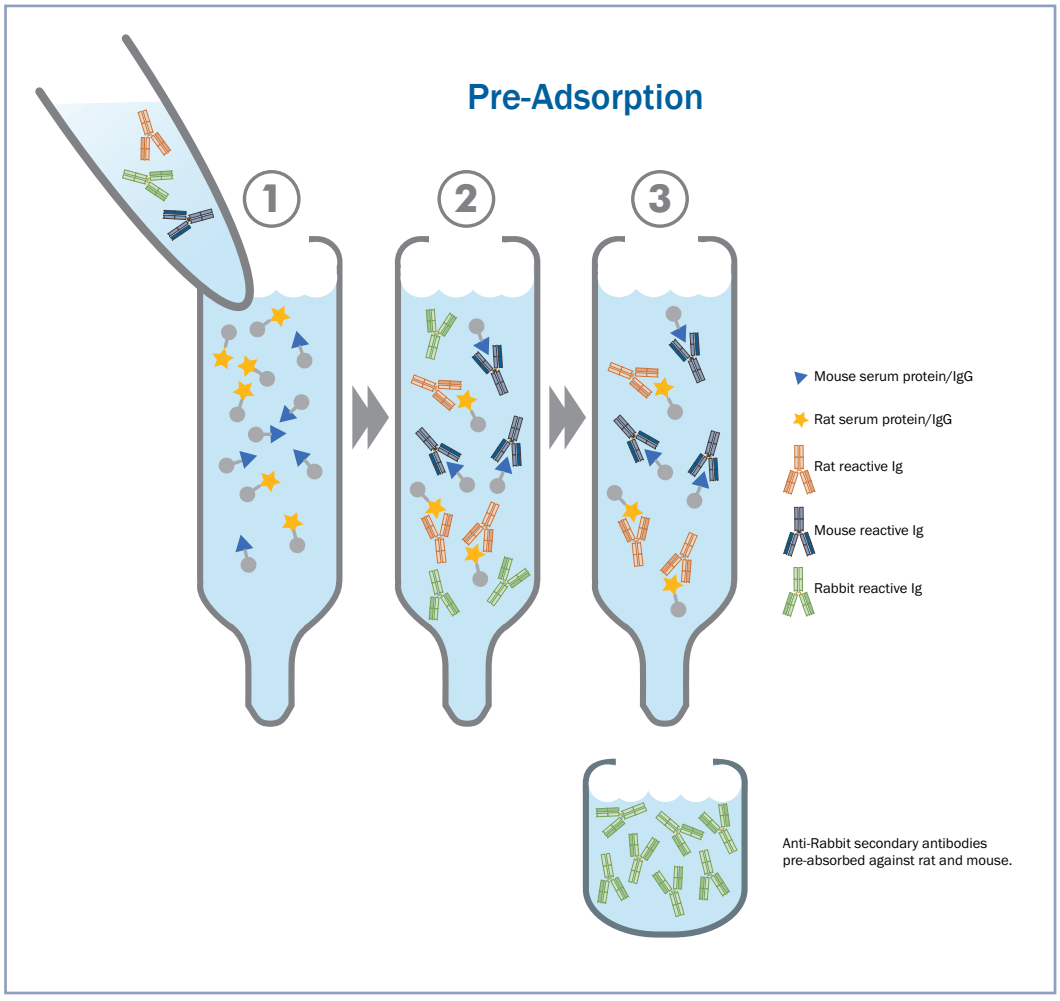
Pre-adsorption is a method to increase secondary antibody specificity and minimize non-specific binding by passing an antibody through a column containing immobilized serum proteins or immunoglobulin from potentially cross-reactive species. Reactive antibodies bind to the immobilized proteins, while non-reactive antibodies flow through (See illustration).

When should I use a pre-adsorbed secondary antibody?

To prevent non-specfic binding, it is recommended to use a pre-adsorbed secondary antibody when determining protein expression in multiple labeling experiments or when staining immunoglobulin-rich tissues or cells. To understand more about pre-adsorption for multiple labeling experiments, please read the Multiple Labeling with Secondary Antibodies section.

Note: A secondary antibody pre-adsorbed against the species of the experimental sample is recommended. For example, a secondary antibody adsorbed against mouse immunoglobulin or serum is recommended when staining mouse tissue.

Secondary Antibody Pre-Adsorption to Limit Cross-reactivity.



In the illustrated pre-adsorption process, rat and mouse immunoglobulin or serum proteins are immobilized on the column. A sample containing rat, mouse, and rabbit secondary antibodies is passed through the column. The antibodies reactive with rat and mouse proteins bind the column, while non-reactive rabbit antibodies pass through. The adsorption process results in rabbit reactive antibodies adsorbed against rat and mouse. These rabbit secondary antibodies are ideal for experiments staining mouse or rat samples.

Secondary Antibodies: Affinity Purified and IgG Fraction Antibodies

Should I use an antigen affinity purified antibody or an IgG fraction?

Whole, unpurified immunoglobulin fractions contain the total complement of antibodies. Whole fractions (usually obtained by Protein A purification) can be further antigen affinity purified to remove antibody subclasses or antigen non-specific antibodies from the sample. Antigen affinity purified secondary antibodies provide less non-specific binding and lower background signal relative to whole IgG fractions. However, the purification process can eliminate high affinity antibodies due to the strong affinity of some antibodies for the binding matrix. Therefore, an IgG fraction is often recommended for low abundance targets.

How are affinity purified antibodies produced?

Due to the high homology of antibody structure between classes, it is recommended class-specific secondary antibodies be affinity purified to minimize cross-reactivity between classes. To produce anti-rabbit IgG affinity purified secondary antibodies, anti-rabbit IgG is passed over a column containing immobilized rabbit IgG. The isolated anti-rabbit IgG antibodies are then passed through an additional column (cross absorption) containing rabbit IgA, IgD, IgE, and IgM proteins. This eliminates antibodies cross-reacting with non-IgG isotpyes (i.e. IgA, IgD, IgE, and IgM). The resulting antigen affinity purified and cross absorbed anti-rabbit IgG secondary antibody should demonstrate minimal reactivity with antibody classes other than IgG.

	Antigen Affinity Purified	Whole Ig Fraction
Sensitivity	Lower sensitivity due to loss of high affinity antibodies during the purification process	Higher sensitivity due to the presence of high affinity antibodies
Specificity	Increased specificity due to elimination of non-specific antibodies	Lower specificity due to presence of non-specific antibodies in the whole Ig fraction
Background	Lower background due to improved specificity	Higher background due to increased non-specific binding
Reproducibility	More reproducible due to lower lot-to-lot variability	Less reproducible due to higher lot-to-lot variability

Secondary Antibodies:
Multiple Labeling with Secondary Antibodies

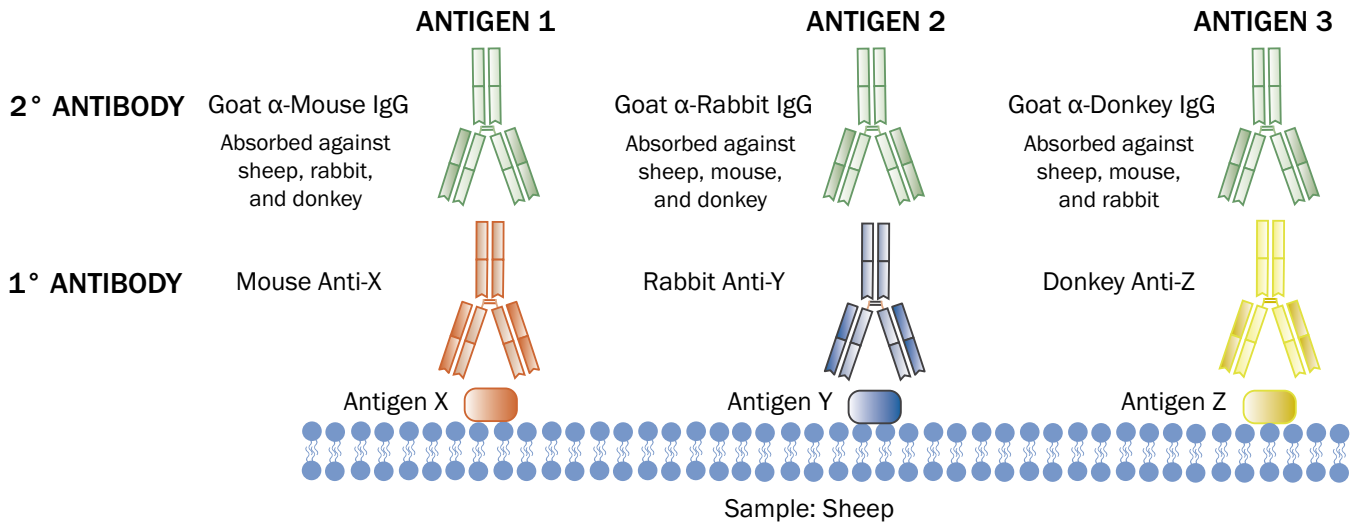
Should I use secondary antibodies raised in the same species in my multiple labeling experiment?

Generating accurate data in double or multiple labeling experiments requires each secondary antibody to specifically recognize one primary antibody. In addition to cross-reacting with primary antibodies, a secondary antibody can bind to the other secondary antibodies or endogenous immunoglobulin expressed in the sample under investigation. Limiting these reactions is key for to producing accurate data.

Multiple Staining with Primary Antibodies Raised in Unique Species

To simultaneously detect multiple proteins in the same sample, all secondary antibodies should be raised in the same host species and adsorbed against the species of all other primary antibodies, as well as the species of the sample being assayed. Secondary antibodies raised in the same species should not bind each other. Adsorbing the secondary antibodies against the host species of the other primary antibodies used in the staining experiment, as well as the species of the sample, eliminates secondary antibody cross-reactivity with endogenous immunoglobulins and other primary antibodies.

Multiple Staining with Primary Antibodies Raised in Unique Species



Antigen X

Steps

01 Block: Block with 5% Goat Serum

02 Primary: Mouse α-Antigen X

03 Secondary: Goat α-Mouse

Antigen Y

Steps

04 Primary: Rabbit α-Antigen Y

05 Secondary: Goat α-Rabbit

Antigen Z

Steps

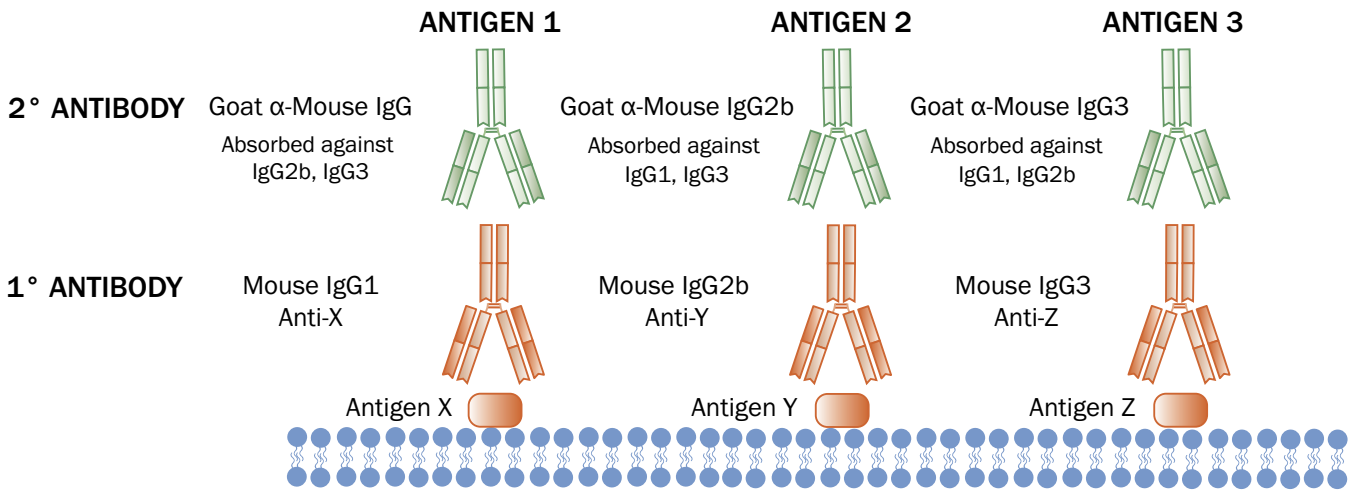
06 Primary: Donkey α-Antigen Z

07 Secondary: Goat α-Donkey

Multiple Staining with Primary Antibodies Raised in the Same Species

In experiments when it is not possible to use primary antibodies from unique species, subclass specific primary antibodies can be used to stain multiple proteins (e.g. IgG1, IgG2b). In this case, subclass specific secondary antibodies pre-adsorbed against the subclasses of all other primary antibodies are recommended.

Multiple Staining with Primary Antibodies Raised in the Same Species



Antigen X

Steps

01 Block: Block with 5% Goat Serum

02 Primary: Mouse α-Antigen X

03 Secondary: Goat α-Mouse IgG1

Antigen Y

Steps

04 Primary: Mouse IgG2b α-Antigen Y

05 Secondary: Goat α-Mouse IgG2b

Antigen Z

Steps

06 Primary: Mouse IgG3 α-Antigen Z

07 Secondary: Goat α-Mouse IgG3

Multiple Labeling for Imaging - Example Color Combinations

	2 Color	3 Color	4 Color
Label 1	DyLight488™	DyLight 488™	DyLight 488™
Label 2	DyLight594™	DyLight594™	DyLight 550™
Label 3		DyLight 647™	DyLight594™
Label 4			DyLight 647™

Dylight™ is a trademark of Thermo Fisher Scientific Inc.

Note: These label combinations are compatible with DAPI nuclear stain. DyLight 488™ can be replaced with DyLight 405™ if DAPI will not be used.

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