Understanding secondary antibodies

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Fragment antigen binding antibodies and isotypes

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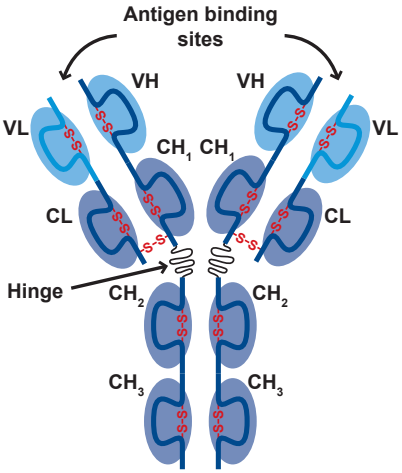
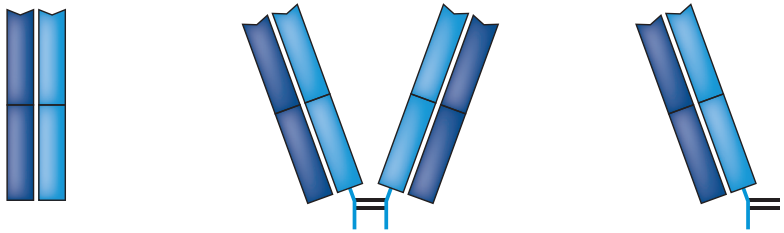
Antibody structure and F(ab) antibodies

The light-chain (LH) folds into a variable domain (VL) and a constant domain (CL), whereas the heavy-chain is composed of one variable domain (VH) and three (IgG and IgA) or four constant domains (IgE).

Antibody structure



F(ab) antibodies



F(ab)
after papain cleavage

F(ab')$_2$
after pepsin cleavage

F(ab')
after pepsin and βmercaptoethanol

The **F(ab) fragment** is an antibody structure that still binds to antigens but is monovalent with no Fc portion (Fc fragments lack all light-chains and the upper part of the heavy-chains). An antibody digested by the enzyme papain yields two F(ab) fragments of about 50 kDa each and an Fc fragment. In contrast, **F(ab')$_2$ fragment** antibodies are generated by pepsin digestion of whole IgG antibodies to remove most of the Fc region while leaving intact some of the hinge region. F(ab')$_2$ fragments have two antigen-binding F(ab) portions linked together by disulfide bonds, and therefore are divalent with a molecular weight of about 110 kDa.

Fragment secondary antibodies

F(ab) fragments against IgG - H&L

Host	Target	Conjugate	Product code
Donkey	Goat	Biotin	ab98827
	Goat	FITC	ab98828
	Goat	HRP	ab98829
	Rabbit	Biotin	ab102281
	Rabbit	FITC	ab102282
Goat	Rabbit	Biotin	ab102285
	Rabbit	FITC	ab102286
	Rabbit	HRP	ab102287
	Rat	Biotin	ab102189
	Rat	FITC	ab102190
	Rat	HRP	ab102191

$F(ab')_2$ fragments

Host	Target	Conjugate	Pre-adsorption	Product code
Donkey	Mouse IgG - H&L	HRP	Hu, Rat, Chk, Cow, Goat, Hrs, Rb, Shp	ab98771
	Rabbit IgG - H&L	HRP	Hu, Ms, Rat, Chk, Cow, Goat, Hrs, Shp	ab98493
	Rabbit IgG - H&L	FITC	-	ab98439
Goat	Mouse IgG - F(ab)	TRITC	Hu, Cow, Hrs	ab51379
	Mouse IgG - Fc	HRP	-	ab98648
	Human IgG - Fc	FITC	Ms, Rat	ab98594
	Human IgG - $(Fab')_2$	Biotin	Ms, Rat	ab98599
	Human IgG - $(Fab')_2$	PE	Ms, Rat	ab98606
	Human IgG - Fc	HRP	-	ab98530
	Rabbit IgG - Fc	Biotin	Hu, Ms, Rat	ab98479
	Rabbit IgG - H&L	FITC	Hu, Ms, Rat	ab98475
	Rabbit IgG - H&L	HRP	Hu, Ms, Rat	ab98476
	Rat IgG - H&L	FITC	-	ab98358
	Rat IgG - H&L	FITC	Hu, Ms	ab98400
Rabbit	Mouse IgG - H&L	Biotin	-	ab98668
	Goat IgG - H&L	HRP	Hu, Ms, Rat	ab102354
Sheep	Mouse IgG - H&L	Cy3®	Hu	ab50502
	Rabbit IgG - H&L	Cy3®	-	ab50503

When to use fragment antibodies

Anti-IgG, Fc fragment secondary antibodies:

(e.g. Goat secondary to Rabbit IgG – Fc (HRP), ab97200)

This antibody cannot bind an antigen as all lights-chains and the upper part of the heavy-chains are missing. IgG Fc fragment antibodies are very specific as they do not react with light-chains or heavy-chains of non IgG antibodies (such as IgA, IgD, IgE and IgM).

F(ab) fragment secondary antibodies:

(e.g. Donkey F(ab) secondary to Rabbit IgG H&L, ab102280)

In comparison to whole IgG and $F(ab')_2$ fragment antibodies, F(ab) fragment antibodies only have one antigen binding site. These antibodies are mainly used for blocking endogenous immunoglobulins on cells/tissues and for double labeling experiments in which researchers would like use primary antibodies from the same host species.

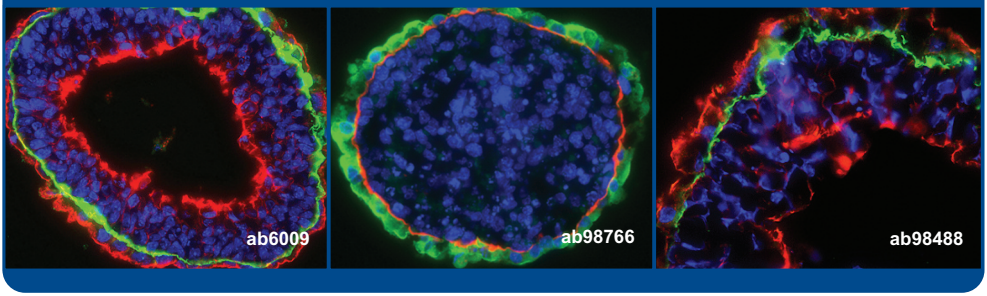
$F(ab')_2$ fragment secondary antibodies:

(e.g. Donkey $F(ab')_2$ secondary to Rabbit IgG H&L (FITC), ab98439)

This antibody type lacks most of the Fc region of the whole IgG molecule. Due to this $F(ab')_2$ fragment antibodies are smaller than whole IgG antibodies and hence can penetrate tissues easier. This is a definite advantage in applications such as immunohistochemistry where $F(ab)_2$ fragment antibodies are used commonly (increased penetration often means increased antigen recognition and increased immunohistochemistry signals). Since IgG $F(ab')_2$ fragment antibodies react with light-chains these antibodies do not exclusively bind to IgG (other immunoglobulins sharing the same light-chain as IgG are also recognized). These antibodies are mainly used in double labeling experiments or when tissues/cells containing Fc receptors are used (e.g. thymus and spleen).

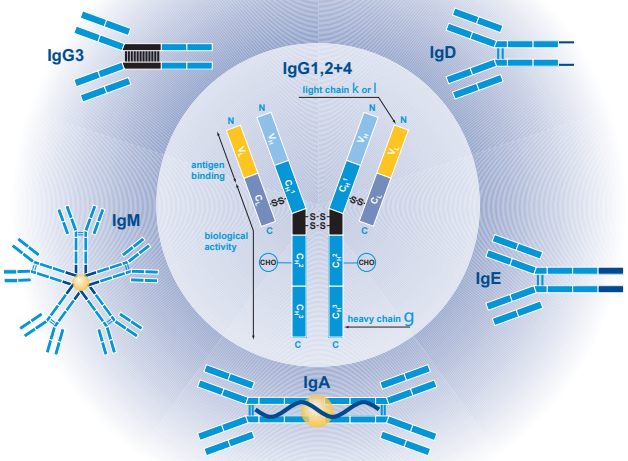
Images are courtesy of Abreviews from Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School.

Examples of $F(ab')_2$ fragment secondary antibodies in multi-color immunohistochemistry



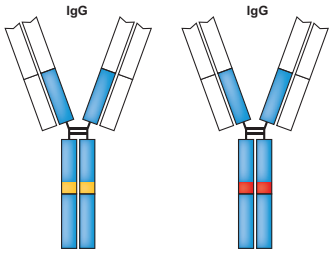
Ig structural differences

Isotypes

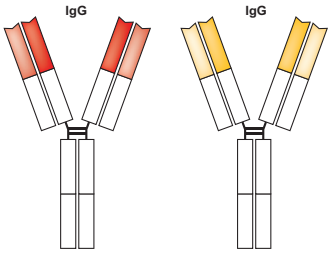


Isotypes: Distinct forms of light and heavy-chains, which are present in all members of a species. Kappa and lambda are isotypes of light-chains; mu, delta, gamma, alpha and epsilon are isotypes of heavy-chains.

Allotypes



Idiotypes

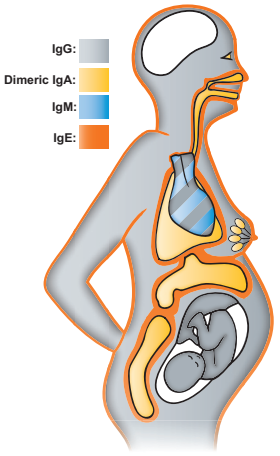


Allotype: Allelic variant within the constant region of the immunoglobulin light or heavy-chains. Of a given isotype, members of a species differ in function to the particular alleles they have received from their parents.
Idiotype: Antigenic specificity of a particular monoclonal immunoglobulin.

Function and distribution of the different Ig isotypes

Ig isotype	Main function	Main structure*
IgG	Predominant immunoglobulin in the secondary immune response; IgG activates the complement system; "opsonizes" bacteria, which makes phagocytosis easier; only immunogloublin that can cross the placenta	Monomer
IgA	Present in secretions such as tears and saliva; IgA prevents microorganisms like bacteria from attaching to mucous membranes	Dimer
IgM	Predominant immunoglobulin in the primary immune response; IgM activates the complement system; it acts as an antigen receptor on the surface of B cells	Pentamer
IgD	Function is elusive; involved in the activation of B cells, basophils and mast cells	Monomer
IgE	Main role is in the defense against worm infections; IgE mediates an immediate hypersensitivity/allergic reaction by triggering the release of chemical mediators such as histamines from mast cells and basophils upon antigen exposure	Monomer

Ig distribution in the human body



Ig isotypes were listed according to the percentage they contribute to the total immunoglobulin present in serum
*Schematic drawings of the Ig isotypes can be seen on the previous page

Related secondary antibodies

Anti-IgG H&L

Host	Target	Conjugate	Pre-adsorption	Product code
Goat	Mouse	AMCA	-	ab47052
	Mouse	Cy3®	Hu, Rat, Chk, Cow, Goat, Hrs, Rb, Shp	ab97035
	Mouse	HRP	-	ab97023
	Rabbit	AP	-	ab81053
	Rabbit	Cy5®	Hu, Ms, Rat, Chk, Cow, Hrs, Pig	ab97077
	Rabbit	PE	Hu	ab50677
	Rabbit	FITC	-	ab97050
	Rabbit	TRITC	Hu	ab50598
	Rat	Biotin	-	ab97055
Rabbit	Mouse	Biotin	-	ab97044
	Mouse	FITC	-	ab97045
	Mouse	HRP	-	ab97046
	Goat	AMCA	-	ab51438
	Goat	TRITC	-	ab50623

Anti-IgA

Host	Target	Conjugate	Pre-adsorption	Product code
Goat	Mouse	HRP	-	ab97235
	Mouse	FITC	-	ab97234
	Mouse	AP	-	ab97232
	Mouse	Biotin	-	ab97233
	Human	HRP	Ms, Rat	ab98558
Mouse	Human	HRP	-	ab99801
Rabbit	Human	HRP	-	ab97220
	Human	FITC	-	ab97219

Anti-IgM

Host	Target	Conjugate	Pre-adsorption	Product code
Goat	Human	Agarose	Ms, Rat	ab65868
	Human	Biotin	-	ab49655
	Human	HRP	Ms, Rat	ab49690
	Mouse	FITC	-	ab97229
	Rabbit	HRP	-	ab97195
Mouse	Rat	FITC	-	ab11679
Rabbit	Human	HRP	-	ab97210
Rat	Mouse	FITC	-	ab11660

Anti-IgD

Host	Target	Conjugate	Pre-adsorption	Product code
Mouse	Human	AP	-	ab99753
	Human	Biotin	-	ab99755
	Human	FITC	-	ab99752
	Human	HRP	-	ab99754
	Human	PE	-	ab99756

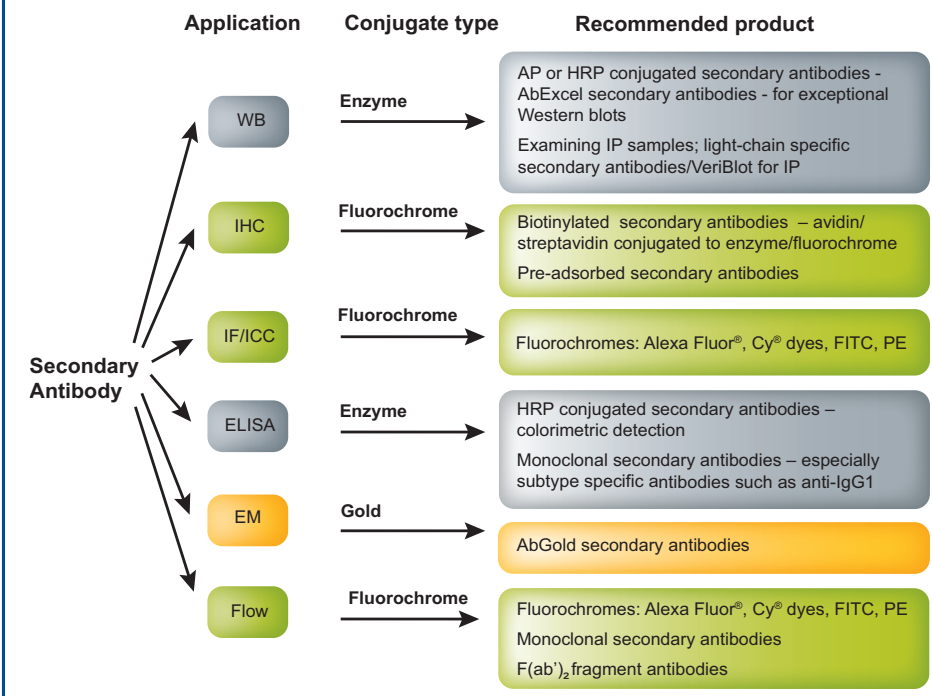
Anti-IgE

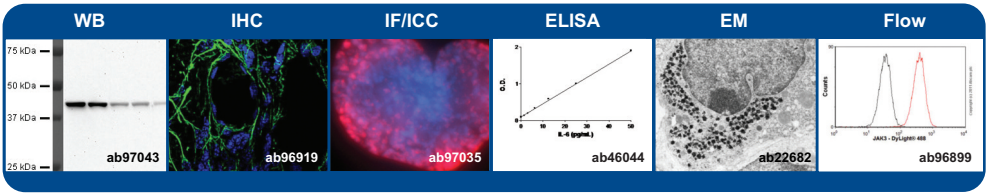
Host	Target	Conjugate	Pre-adsorption	Product code
Mouse	Human	AP	-	ab99805, ab99835
	Human	Biotin	-	ab99807, ab99837
	Human	HRP	-	ab99806, ab99836
	Rat	Biotin	-	ab11666
	Rat	FITC	-	ab11667

Abbreviations: Chk = Chicken, Hrs = Horse, Hu = Human, Ms = Mouse, Rb = Rabbit, Shp = Sheep, ELISA = Enzyme Linked Immunosorbent Assay, EM = Electron Microscopy, IF/ICC = Immunofluorescence/Immunocytochemistry, IHC = Immunohistochemistry, Flow = Flow Cytometry, WB = Western blot

Conjugate selection guide

Secondary antibody applications





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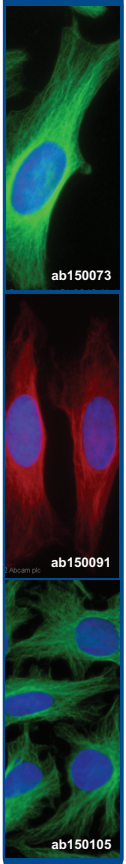
ab96919: this image is courtesy of an Abreview by Carl Hobbs, King's College London. ab97035: image is courtesy of an Abreview by Dr. Kirk McManus, Univ. Of Manitoba/Cancer Care MICB, Canada. ab22682: this image is courtesy of an anonymous Abreview.

Fluorescent detection methods

Spectral properties of fluorochromes available from Abcam

Fluorochrome name	Excitation (nm)	Emission (nm)	Color
AMCA (Aminocoumarin)	350	445	Blue
Allophycocyanin (APC)	650	660	Red
Chromeo™488	488	517	Green
Chromeo™ 494	494	628	Red
Chromeo™ 505	505	526	Green
Chromeo™ 546	545	561	Yellow
Chromeo™ 642	642	660	Red
Cy2®	489	506	Green
Cy3®	550	570	Yellow
Cy3.5®	576	589	Red-orange
Cy5®	649	670	Red
Cy5.5®	675	694	Far red/near IR
Alexa Fluor® 488	495	519	Green
Alexa Fluor® 555	555	565	Yellow
Alexa Fluor® 594	590	617	Red-orange
Alexa Fluor® 647	650	665	Red
FAM	495	520	Green
FITC (Fluorescein Isothiocyanate)	495	528	Green
PE (Phycoerythrin)	496 and 545/566	576	Orange-yellow
SureLight® P1	545	666	Red
SureLight® P3	614	662	Red
SureLight® PE	480	578	Yellow
Spectral Red (SPRD) (PE/Cy5®)	565	666	Red
Texas Red®	490,675	695	Red
TRITC (Tetramethyl Rhodaminelsothiocyanate)	557	576	Orange-yellow

Alexa Fluor®



More information about how fluorochromes work can be found at www.abcam.com/fluorescentguide

Discover more at abcam.com/fluorochromes

How to choose the right secondary

Guide to help you choose the most appropriate secondary antibody for your application.

1. What is the host species of the primary antibody?
The secondary antibody is directed against the species of the primary antibody. If you use a primary antibody raised in rabbit, you will need an anti-rabbit secondary antibody raised in a species other than rabbit.

2. What do I need to know about the isotype of the primary 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 an IgG isotype. The secondary antibody will typically be an anti-IgG H&L 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 the 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-chains

3. Do I need an enzymatic or fluorescent detection?
The type of conjugation is application dependent.
For enzymatic and biotin detection, e.g. in WB or ELISA, we suggest a secondary antibody

antibody?

conjugated to HRP, AP or biotin. Both avidin and streptavidin bind very strongly to biotin and enable signal amplification, regardless of the host species of the antibody.
If a laser light is used, e.g. in Flow Cytometry, ICC/IF or IHC, we suggest fluorescent detection with a secondary antibody conjugated to a fluorochrome.

4. Do I need a pre-adsorbed secondary antibody?
We usually recommend using a secondary antibody, pre-adsorbed with serum, for western blotting, of immunoglobulin-rich tissues and cells. Pre-adsorbed secondary antibodies are less likely to interact with endogenous immunoglobulins and consequently may reduce non-specific background. The secondary antibody should be pre-adsorbed against the same species as the sample on which the detection is performed. For example, a human pre-adsorbed antibody will be required for detection in human tissue.

5. Do I need an affinity purified antibody or IgG fraction?
The advantage of using affinity purified antibodies or IgG fractions will depend on the type of binding expected. Affinity purified antibodies give the lowest amount of non-specific binding whereas IgG fractions contain high affinity antibodies. Indeed, during an affinity purification, high affinity antibodies stay fixed on the matrix and cannot be eluted.

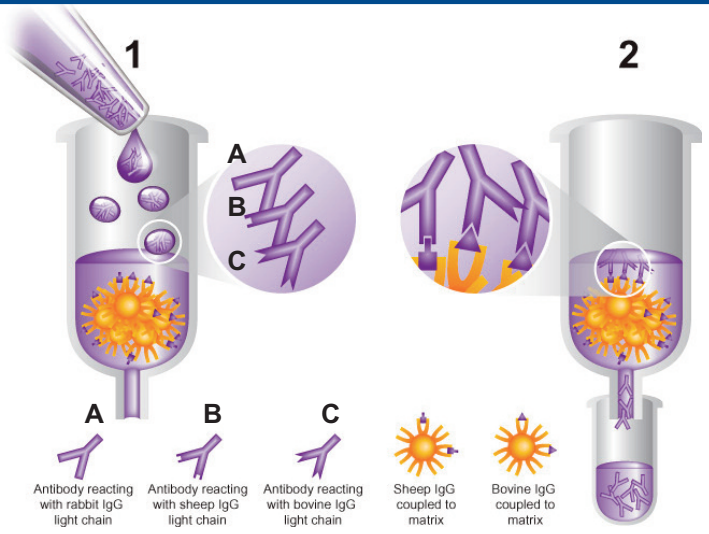
6. Is it necessary to use F(ab')$_2$ fragment antibody?
F(ab')$_2$ fragment antibodies eliminate non-specific binding between Fc portions of antibodies and Fc receptors on cells (such as macrophages, dendritic cells, neutrophils, NK cells and B cells) and penetrate tissues more efficiently due to their smaller size. As fragment antibodies do not have Fc portions, they do not interfere with anti-Fc mediated antibody detection.

7. Do I need an anti-IgG H&L, anti light-chain or anti-F(ab')$_2$ secondary antibody?
Our secondary antibodies are supplied in different formats:
- Anti-IgG H&L antibodies react with both heavy and light-chains of IgG subclasses
- Anti light-chain antibodies react with the light-chain of primary antibodies which is the same among all classes
- Anti-F(ab')$_2$ secondary antibodies react with the F(ab')$_2$ portion of the primary antibody

Pre-adsorbed secondary antibodies

Pre-adsorbed secondary antibodies are ideal for multi-color imaging and immunohistochemistry experiments when several primary antibodies and their corresponding secondary antibodies are used simultaneously.

Pre-adsorption process



Pre-adsorption (or cross-adsorption) is an extra step to increase the specificity of an antibody. Pre-adsorption process: **Step 1.** Antibodies, such as the mixture of antibodies recognizing rabbit IgG light-chains (A,B,C) shown in the above diagram, are passed through a matrix containing immobilized serum proteins. These proteins are from potentially cross-reactive species; in the above drawing, pre-adsorption against sheep and bovine was required and therefore serum proteins recognizing sheep and bovine light-chains were bound to the matrix. **Step 2.** Only antibodies specific to rabbit IgG light-chains (A) will pass through the column. Antibodies cross-reacting with sheep or bovine IgG light-chains (B and C) will bind and stay adsorbed to the matrix.