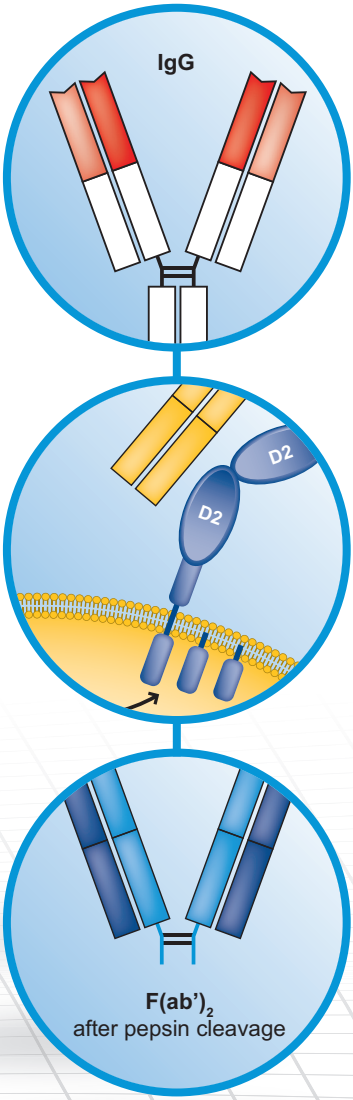
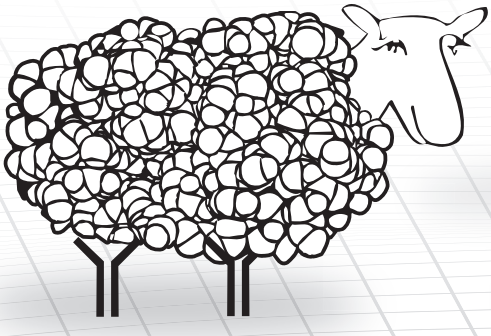
abcam®

Understanding secondary antibodies

Fragment antigen binding antibodies and isotypes

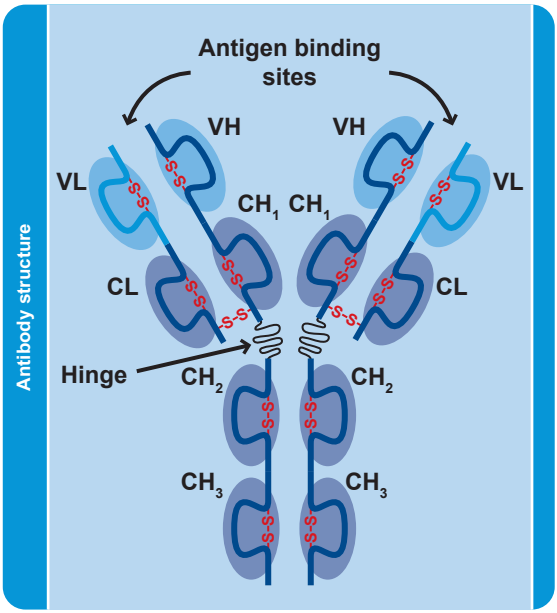


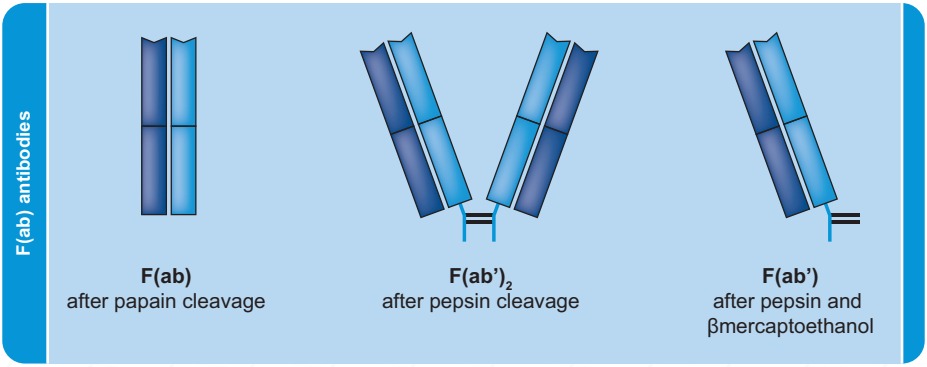


www.abcam.com/secondary_antibody

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).





The **F(ab) fragment** is an antibody structure that still binds to antigens but is monovalent with no Fc portion. 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.

Secondary antibodies

F(ab) fragments

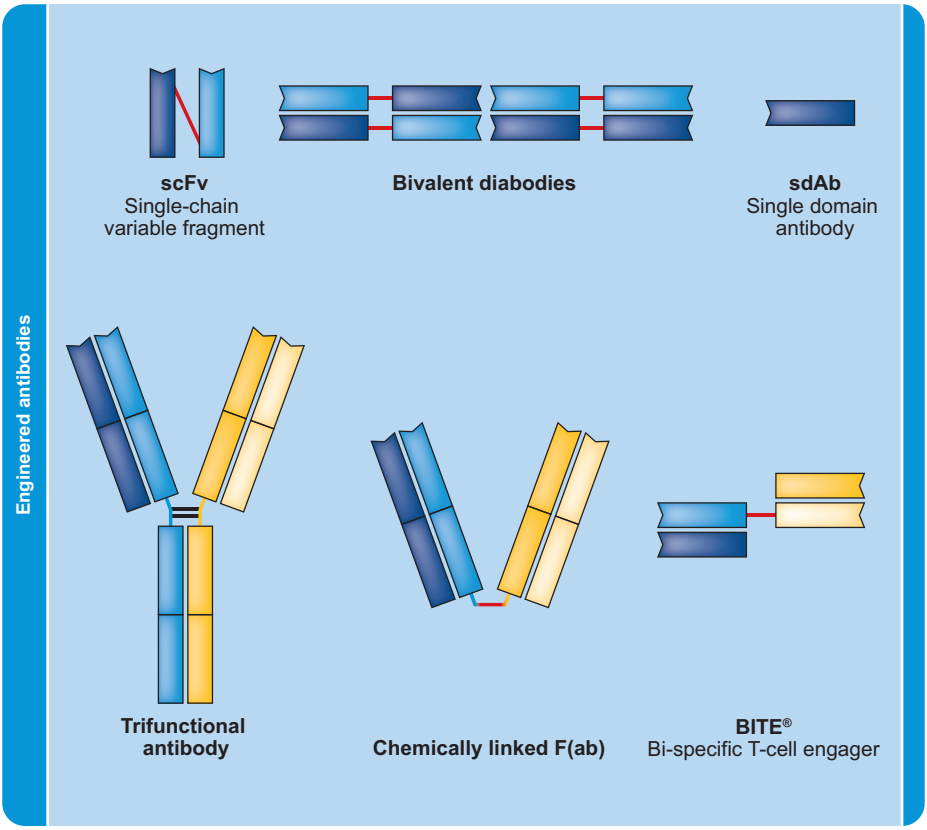
Host	Target	Conjugate	Applications	www.abcam.com/…
Donkey	Goat IgG - H&L	HRP	Dot, ELISA, EM, IHC, WB	ab6667
	Goat IgG - H&L	TRITC	Flow Cyt, IM	ab6522
	Goat IgG - H&L	TR	Flow Cyt, IM	ab6523
	Goat IgG - H&L	Biotin	Dot, ELISA, IHC, IM, WB	ab6578
Goat	Human IgG - H&L	1.4nm Gold	EM, WB	ab30826
	Mouse IgG - F(ab'), pre-adsorbed	TRITC	Flow Cyt, ICC/IF, IHC-P	ab51379
	Mouse IgG - H&L			ab6668
	Mouse IgG - H&L	HRP	Dot, ELISA, EM, IHC, WB	ab6823
	Mouse IgG - H&L	TRITC	Flow Cyt, IM	ab6670
	Mouse IgG - H&L	FITC	Flow Cyt, IM	ab6669
	Rabbit IgG - H&L			ab6824
	Rabbit IgG - H&L	Biotin	Dot, ELISA, IHC, IM, WB	ab7055
	Rabbit IgG - H&L	HRP	Dot, ELISA, EM, IHC, WB	ab7171
	Rat IgG - H&L	Biotin	Dot, ELISA, IHC, IM, WB	ab7176

$F(ab')_2$ fragments

Host	Target	Conjugate	Applications	www.abcam.com/…
Donkey	Goat IgG - H&L, pre-adsorbed	PE	Flow Cyt, IHC-Fr, IHC-P, IM	ab7004
	Rabbit IgG - H&L, pre-adsorbed	PE	Flow Cyt, ICC/IF, IHC-P, IM	ab7007
Goat	Mouse IgG - $(Fab)'_2$, pre-adsorbed	TR	Flow Cyt, IM	ab5884
	Mouse IgG - Fc, pre-adsorbed	PE	Flow Cyt, IM	ab5881
	Mouse IgG - H&L, pre-adsorbed	PE	Flow Cyt, IHC-Fr, IHC-P, IM	ab7002
	Mouse IgG+IgM+IgA - H&L, pre-adsorbed	HRP	Dot, ELISA, EM, IHC, WB	ab6006
	Mouse IgG+IgM+IgA - H&L, pre-adsorbed	FITC	Flow Cyt, IF	ab5999
	Mouse IgM - mu chain	Biotin	Dot, ELISA, Flow Cyt, IHC, IM, WB	ab5929
	Mouse IgM - mu chain	HRP	Dot, ELISA, EM, IHC, IHC-Fr, WB	ab5930
	Mouse IgM - mu chain	AP	Dot, ELISA, IHC, WB	ab5931
	Mouse IgM - mu chain	FITC	Flow Cyt, IM	ab5926
	Rabbit IgG - $(Fab)'_2$, pre-adsorbed	HRP	Dot, ELISA, EM, IHC, WB	ab6112
	Rabbit IgG - $(Fab)'_2$, pre-adsorbed	TRICT	Flow Cyt, IM	ab6110
Rabbit	Goat IgG - H&L	HRP	Dot, ELISA, EM, IHC, WB	ab5755
	Goat IgG - H&L	FITC	Flow Cyt, ICC/IF	ab47846
Sheep	Mouse IgG - H&L , pre-adsorbed	Cy3®	IHC-P	ab50502

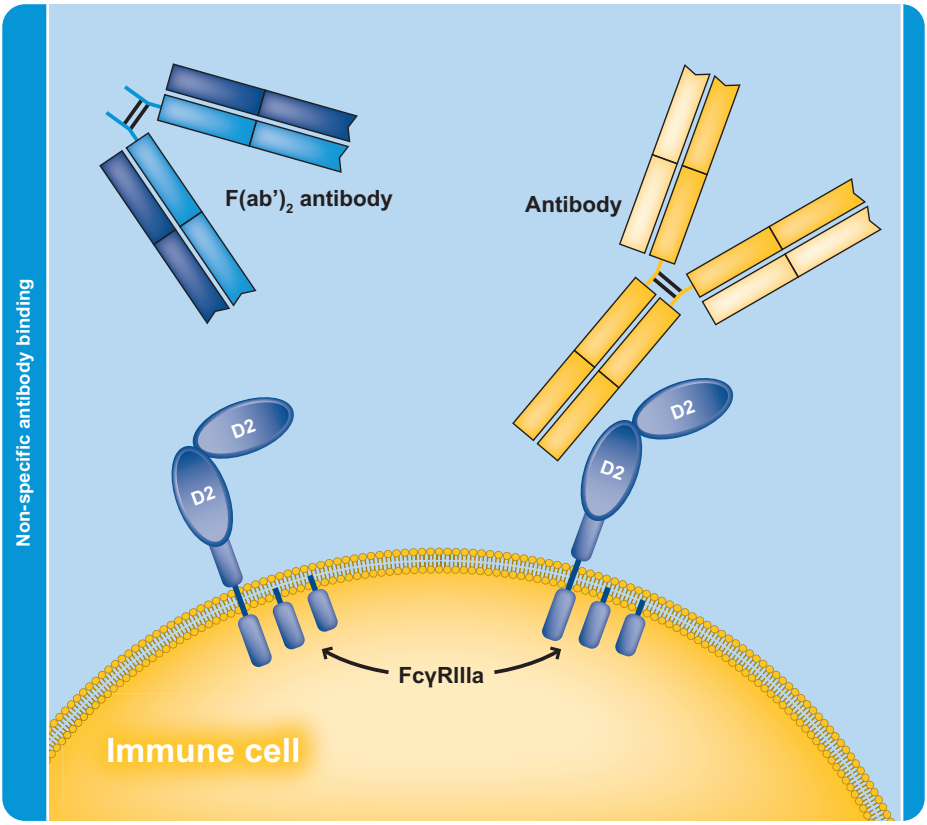
See more species and isotype reactivity at **www.abcam.com/search_secondary**

Engineered antibodies



- A **single-chain variable fragment** (scFv) is a powerful tool in phage display, as it can express the antigen-binding domain as a single peptide. ScFv is also used in FACS, IHC and as antigen-binding domains of artificial T cell receptors.
- **Diabody** plays a role in the recruitment of antibody effector functions and cytotoxic T-cell responses.
- A **single domain antibody** (sdAb) consists of a single monomeric variable antibody domain and is involved in pharmaceutical applications.
- A **trifunctional antibody** is a monoclonal antibody with binding sites for two different antigens, typically CD3 and a tumor antigen. Trifunctional antibodies are considered as a type of bispecific monoclonal antibody.
- **Bi-specific T-cell engagers** (BiTE®) is a class of bispecific monoclonal antibody that direct a host's T cells cytotoxic activity against diseased cells.

F(ab) and Fc Receptors

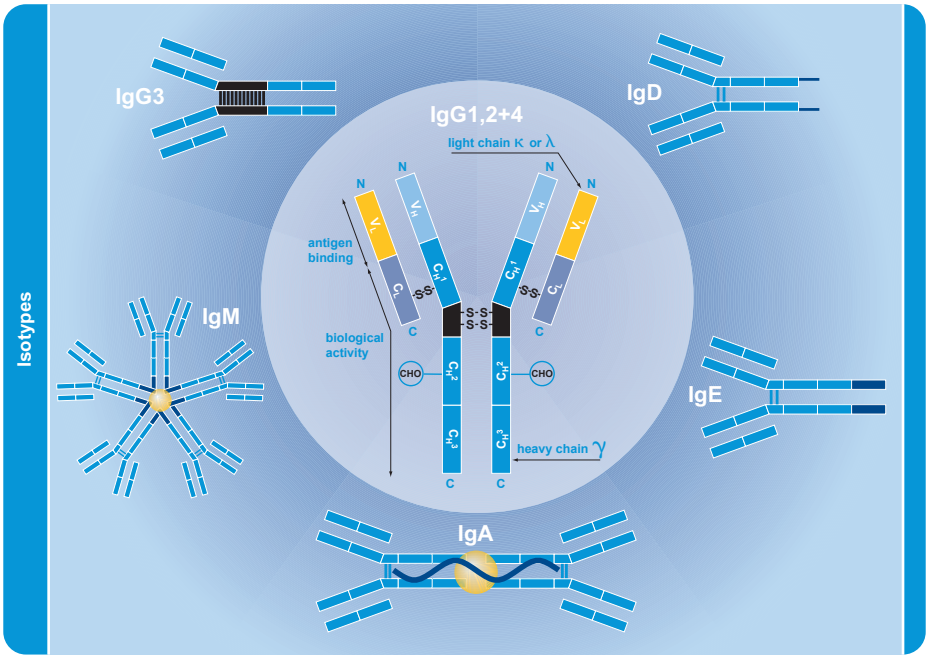


Fc receptor names

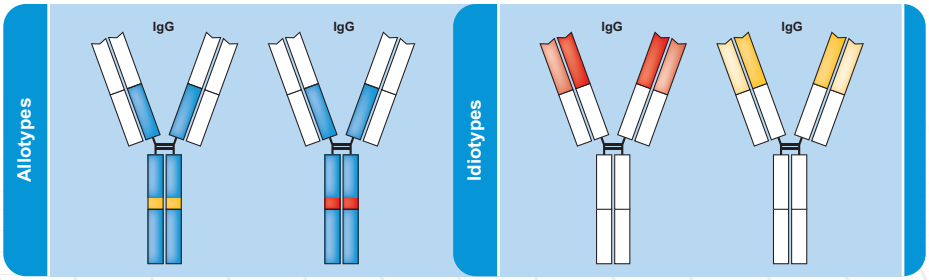
FcγRI.....CD64	**FcγRIIB2**.....CD32	**FcεRII**.....CD23
FcγRIIA.....CD32	**FcγRIIIA**.....CD16a	**FcαRI**.....CD89
FcγRIIB1.....CD32	**FcγRIIIB**.....CD16b	

F(ab) and F(ab')$_2$ antibodies eliminate non-specific binding between Fc portions of antibodies and Fc receptors on cells (such as macrophages, dendritic cells, neutrophils, NK cells, B cells…)

Ig structural differences



Isotype: 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.



Allotype: Allelic variants 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.

Secondary antibodies

Anti-IgG H&L

Host	Target	Conjugate	Applications	Datasheet www.abcam.com/…
Donkey	Goat	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6885
Goat	Mouse	FITC	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IM, WB	ab6785
	Mouse	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6789
	Mouse	TRITC	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IM	ab6786
	Rabbit	AP	Dot, ELISA, ICC/IF, IHC-Fr, IHC-P, WB	ab6722
	Rabbit	Biotin	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6720
	Rabbit	Cy3®	Flow Cyt, ICC, IF, IHC-Fr, IHC-P	ab6939
	Rabbit	Cy5®	Flow Cyt, ICC, IF, IHC-Fr, IHC-P	ab6564
	Rabbit	FITC	Flow Cyt, ICC/IF, IHC-Fr, IHC-P, IM	ab6717
	Rabbit	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6721
Rabbit	Goat	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6741
	Mouse	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6728
	Mouse	TR	Flow Cyt, IHC-Fr, IHC-P, IM	ab6726
	Rat	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6734
	Sheep	HRP	Dot, ELISA, IHC-Fr, IHC-P, IM, WB	ab6747

Anti-IgM

Host	Target	Applications	Datasheet www.abcam.com/…
Goat	Human	Dot, ELISA	ab8496
	Human	ELISA, ID, Ie, WB	ab9160
	Mouse	ELISA, ID, Ie, WB	ab9167
	Mouse (Biotin)	ELISA	ab49760
	Rabbit	ELISA, ICC, WB	ab2754
Mouse	Sheep (clone [G9])	Conjugation, ELISA	ab19991
Rabbit	Human	Dot, ELISA	ab8505
	Mouse	ELISA, Ie, WB	ab9175
Rat	Rabbit (clone [SB87a])	ELISA, Flow Cyt	ab80301

Anti-IgA

Host	Target	Applications	Datasheet www.abcam.com/…
Chicken	Human	ELISA, ID, Ie, WB	ab9151
Goat	Human	Dot, ELISA	ab8499
	Human	ELISA, ID, Ie, WB	ab9157
	Mouse	ELISA, ID, Ie, WB	ab9161
	Rabbit	ELISA, ICC, WB	ab2758
Mouse	Human (clone [hIgA1])	ELISA, RIA	ab433
Rabbit	Human	Dot, ELISA	ab8508
	Mouse	ELISA, ID, Ie, WB	ab9170

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Enzymatic detection methods

Enzyme	Substrates	Applications	Advantages	Disadvantages
Horseradish Peroxidase (HRP)	Chromogenic, soluble (TMB, ABTS, OPD...)	ELISA	Easy to use	Light sensitive coloration
	Chromogenic, precipitating (CN, AEC, DAB,...)	WB, SB, IHC	Easy to use	Background in blood samples and some other tissues Staining stability lower than AP
	Fluorogenic (ADHP/resorufin) Chemiluminescent	ELISA	High sensitivity	Need fluorescence equipment
	Luminol	WB, SB, IHC	High sensitivity	Need radiographic equipment or light scanner
Alkaline Phosphatase (AP)	Chromogenic, soluble (pNPP)	ELISA	Linear kinetic Often more sensitive than HRP	Unstable
	Chromogenic, precipitating (BCIP/NBT,...)	WB, SB, IHC	Staining stability higher than HRP	Interference with nuclear counterstain
	Fluorogenic (4-MUP)	ELISA, IHC	Sensitivity	Need fluorescence equipment

WB: Western Blot, SB: Southern Blot, IHC: Immunohistochemistry,
ELISA: Enzyme Linked Immunosorbent Assay

How to choose the right seconda

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 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 conjugated to HRP, AP or biotin. Both avidin and streptavidin bind very strongly to biotin and enable signal amplification,

ry antibody?

regardless of the host species of the antibody.
If a laser light is involved, 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 immunolgobulins 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 a F(ab) or F(ab')$_2$ fragment antibody?
F(ab) and 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

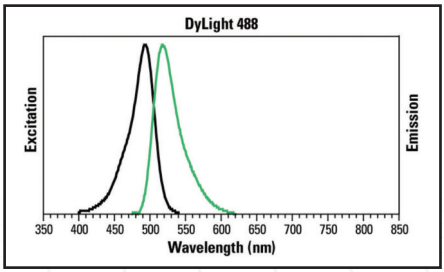
For more information and links to all our downloads please visit: **www.abcam.com/secondary_antibody**

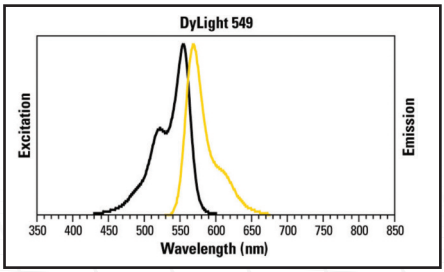
Fluorescent detection methods

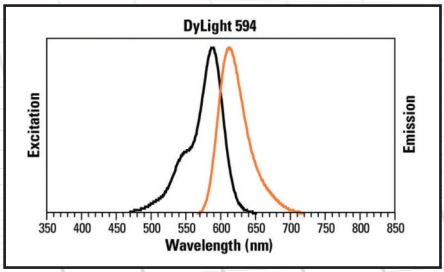
Spectral properties of DyLight® fluorochromes

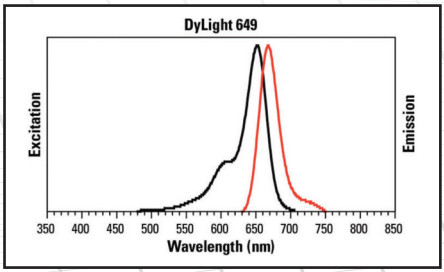
Fluorochrome	Color	Ex/Em	ε ($M^{-1}cm^{-1}$)	Spectrally equivalent dyes
DyLight® 488		493/518	70K	Alexa Fluor 488®, Fluorescein, FITC, Cy2®
DyLight® 549		562/576	150K	Alexa Fluor 546® or 555®, Cy3®, TRITC
DyLight® 594		593/618	80K	Alexa Fluor 594®, Texas Red®
DyLight® 649		654/673	250K	Alexa Fluor 647®, Cy5®

Ex/Em: Excitation and emission wavelength in nanometers (+/- 4nm)
ε: Molar extinction coefficient at the absorption maximum









More information at **www.abcam.com/DyLight**

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100% technical support

- Thinking of purchasing a secondary antibody but have some technical questions?
- Unsure of buffers or incubation times?
- Having difficulty with a product? *...Our scientific experts are here to help.*

All Abcam secondary antibodies are guaranteed to work as specified on the datasheet.

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- We guarantee our products work in the tested species and applications as stated on the datasheet
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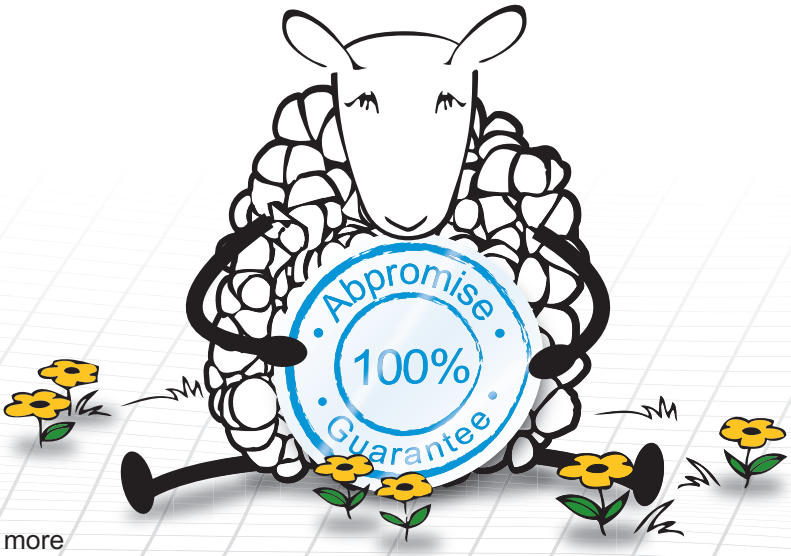

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