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Content

Antibody Structure

IgG as a reference structure for Immunoglobulins

Immunoglobulin G (IgG) consists of two identical „L"ight and „H"eavy polypeptide chains that are connected through covalent disulfide bonds.

The lower segment is also known as Fc-Fragment (fragment crystallizable). Two "arms" of the upper part of the molecule consist of part of the H-Chain and one L-chain that is also known as Fab-Fragment (antigen binding fragment).

The light chain consists of one variable (VL) and one constant (CL) domain, each. In contrast, a heavy chain consists of one variable and three constant domains that are known as VH, CH1, CH2 and CH3.

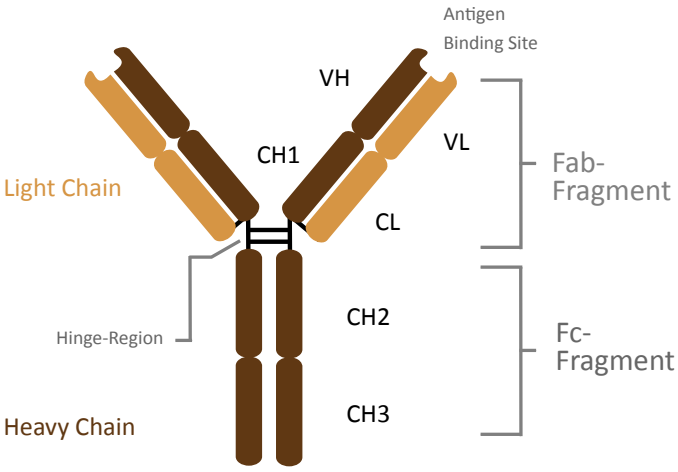


Fig. 1: Schematic of Immunoglobulin G (IgG)

Additional Immunoglobulins / Immunoglobin Classes

Mammals have 5 immunoglobulin classes (isotypes) namely IgG, IgA, IgM, IgE and IgD.

Differences between isotypes are located on the heavy chains. IgM and IgE have an additional constant domain (CH4) in addition to variations in the peptide sequence compared to the 3 constant domains of the other isotypes. IgA is expressed as a homodimer, joined via a linker peptide that is called Joining Chain (J chain) or Joining Peptide, wheras IgM is expressed as pentamer that also contains a J Chain.

In addition, IgG and IgA can be grouped in 4 and 2 subclasses, respectively, that show lesser variations in the peptide sequence compared to the main classes.

Light Chain Types Kappa (κ) and Lambda (λ)

Differences between classes and subclasses are only found on the heavy chains. But there are also two types of light chains that are called kappa (κ) and lambda (λ), where κ light chain is more abundant in normal serum.

Structure of Camelide Antibodies

Camelides have a unique IgG subclass that is not found in any other mammal. In addition to conventional IgG1 they express IgG2 and IgG3 that only consist of a CH2 and CH3 domain, but lack a CH1 domain and a light chain. Those heavy chain antibodies have a small size, are very stable and show a high specificity and affinity to the antigen they recognize.

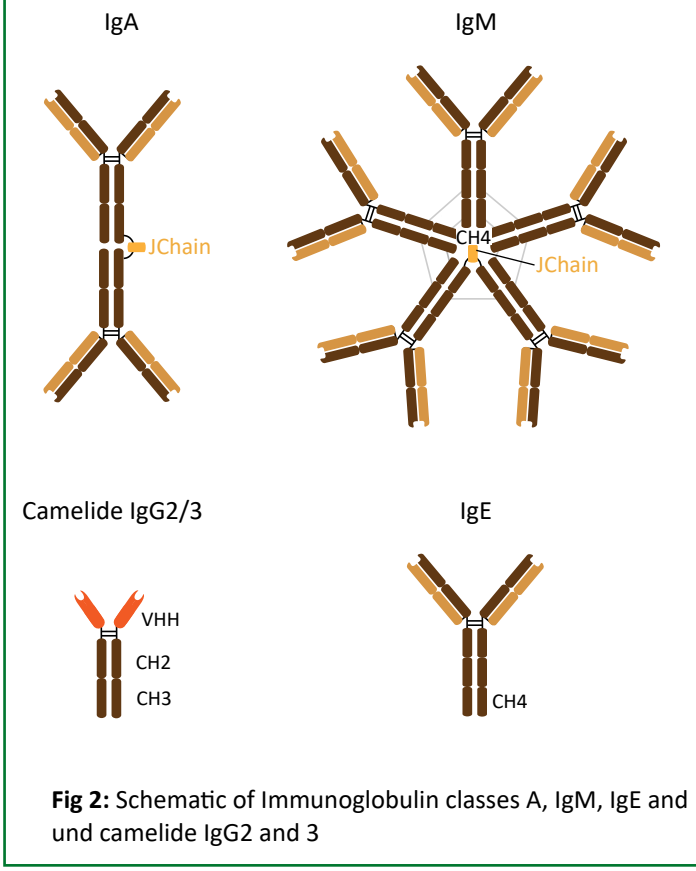


Fig 2: Schematic of Immunoglobulin classes A, IgM, IgE and und camelide IgG2 and 3

What is a Secondary Antibody?

What is a Secondary Antibody?

The term secondary antibody is derived from their functional property to detect another antibody as antigen. In analogy the target is called primary antibody.

Immunodetection using only one antibody is also known as direct labeling / detection. The primary antibody is directly linked to a reporter molecule that can be a fluorescent dye or enzyme that transforms a substrate into a colored reaction product. Antibodies covalently linked to a reporter molecule are also called conjugates.

In contrast, methods that use a secondary antibody to detect a primary antibody are referred to as indirect labeling / detection.

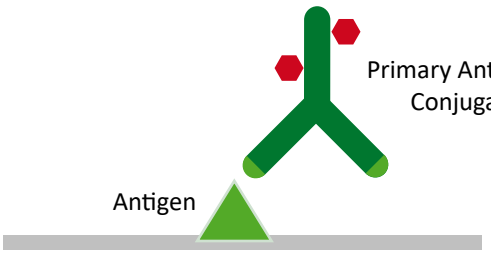
Secondary antibodies are made by immunizing animals with immunoglobulins or parts of immunoglobulins from other species. The used antigen and how the resulting antibody is purified affects the specificity and sensitivity of the secondary antibody.

What are the advantages of Secondary Antibodies compared to direct methods?

In contrast do direct methods secondary antibodies have a number of advantages. More than one secondary can bind to a single primary antibody resulting in a signal enhancement. Secondary antibodies offer more flexibility in every day lab use, because not every single primary antibody needs to be obtained as a primary antibody conjugate.

In multi-labeling experiments all reagents especially the secondary antibodies must be selected very carefully to guarantee accurate results.

Direct Detection



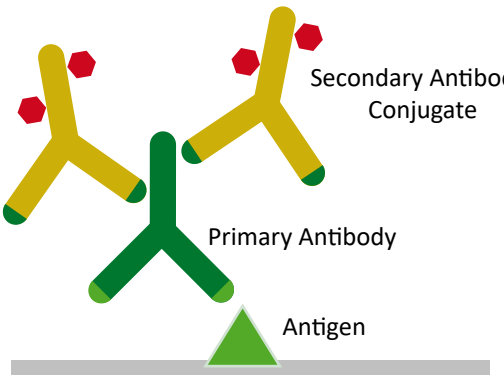
\+ Less time,
due to 1-step protocol

\+ No problems with cross-reactivity
of secondaries in multi-labeling experiments

\- Lower signal strength / lower sensitivity
(no signal enhancement)

\- Low flexibility
each antibody needs to be conjugated

Indirect Detection



\+ High flexibility: one secondary can
detect many primary antibodies

\+ Higher sensitivity / signal enhancement

! Needs careful planning
/ experiment design

\- More time consuming due to
2-step protocol

anti-Mouse

Species Reactivity

Step 1:

What should be the Target Species of the Secondary Antibody?

Generally, the target species is the host species the primary antibody is made in. There are only a few exceptions to this rule when cross-reacting antibodies can be used against related species

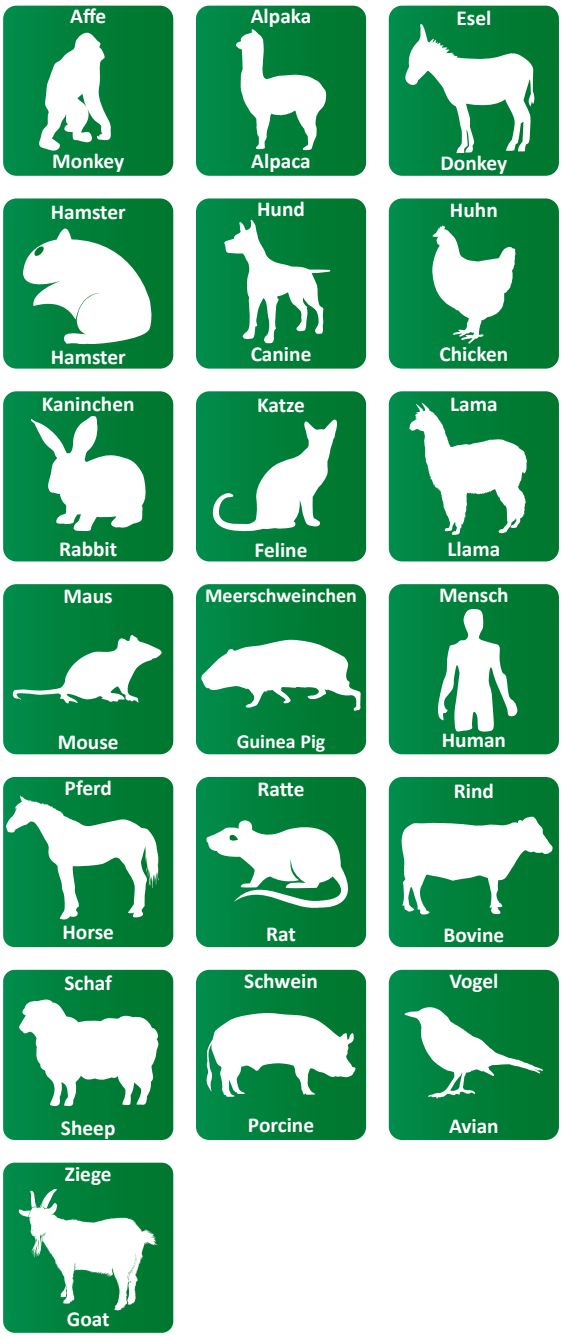
Antibodies against Hamster IgG

Nearly all monoclonal antibodies made in hamster are derived from interspecies mouse: Armenian Hamster hybridoma. Those IgGs are "Armenian" and not "Syrian" Hamster IgG. In contrast, most polyclonal anti-Hamster antibodies are directed against Syrian Hamster IgG that detect monoclonal antibodys from Armenian Hamsters with less sensitivity.

Detection of Antibodies from Sheep, Goat and Bovine

IgGs from sheep, goat and bovine are to a great extent homologous to each other, so that antibodies made against goat IgG can also be used to detect bovine or sheep IgG. Vice versa antibodies against sheep can also be used to detect bovine antibodies or antibodies from goat.

Selection of species reactivity / target species



Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	Format	**Species Reactivity**	Specificity	Conjugation	Adsorbtion

Donkey

Host Species

Step 2:

Which Host should be selected for the Secondary Antibody

From our experience antibodies made in goat or donkey are suitable to detect primary antibodies from most species.

When using indirect methods with unconjugated primary antibodies all secondary antibody conjugates should be from the same host species. This avoids complications due to cross-reactivity between the secondary antibodies.

Generally, highly adsorbed secondary antibodies (see section Adsorbtion) can be considered as more specific but may also be less sensitive for their intended target.

Bovine Antibodies for the detection of Goat IgG

Some reagents that are used in the laboratories may contain bovine immunoglobins. Examples are FCS used as supplement in cell culture media, milk powder used as blocking reagent in Western Blot applications or BSA used as stabilizing reagent for proteins and antibodies.

Trying to detect antibodies from goat in the presence of such reagents can cause problems due to the close relationship between goat and bovine IgG. In order to avoid such problems, we recommend using secondaries of bovine origin from Jackson ImmunoResearch (805-xxx-180).

Antibodies from Alpaca

Antibodies from Alpaca are also suitable as secondaries, when reagents containing bovine IgGs are used. In adition to conventional IgG1, serum of camelides contain IgG2 and IgG3, that do not have any light chains nor a CH1 domain in their heavy chain (also see chapter secondary antibody structure). These heavy chain antibodies have a small size and show a high specificity and affinity to their respective antigen. Those properties make them especially useful as secondary antibodies.

Immunoprecipitation with Rabbit Antibodies

In case antigen-antibody complexes need to be separated from other components using Protein A Agarose, rabbit antibodies are the reagent of choice. Rabbit antibodies have a higher affinity to Protein A compared to IgG from goat, sheep or donkey. For separation of IgG from goat, sheep and donkey (as well as rabbit) Protein G is recommended.

Selection of a suitable secondary antibody host species

- For multiple labeling all secondarys should be from the same host!
- For multiple labeling, every secondary should be adsorbed against all other species Ig that may be present in the experimental setup (s. step Adsorbtion)!
- To reduce background staining, normal serum from the same host species as the secondary antibody should be used as blocking reagent.

Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	Format	Species Reactivity	Specificity	Conjugation	Adsorbtion

F(ab')2

Format

Step 3:

Format of the Secodary Antibody: Whole Molecule (H+L), F(ab')2- or Fab-Fragment?

IgG whole molecule is the most widely used antibody format and is suitable for most applications.

In some applications other formats that can be obtained by enzymatic digestion of IgG whole molecules (see figure below) may be more suitable:

F(ab')2-Fragments

Due to the lack of Fc-Part, F(ab')2-Fragments have less tendency to form aggregates and are, compared to complete IgG, smaller in size. They do not interact with Fc-receptors that can be a cause for background staining. When background staining caused by secondary antibody interaction with Fc-Receptors is an issue or if better diffusion properties of the secondary reagent is required, F(ab')2-Fragments should be considered.

Monovalent Fab-Fragments can be used as blocking reagents and as detection reagents when both primary antibodies originate from the same host. They can also be used as blocking reagents against endogenous Immunoglobulins in stainings where tissue and primary antibody are from the same species. Fab-Fragments have only one antigen binding site and cannot bind additional antibodies

Antisera against IgG whole IgG molecules are recommended as bridging secondary antibodies in PAP an APAAP-Stainings.

Selection of the right antibody format

\+ Optimal for most applications **(bivalent binding)**

\+ Reduced background: No Fc-Receptor binding

\+ Better diffusion: smaller than IgG(H+L)

\+ Good antigen binding: dual antigen binding sites **(bivalent)**

\+ Suitable for special methods: Only one antigen binding site **(monovalent)**

\- Due to lower affinity not recommended for standard methods

! Attention, usage of F(ab')2-Fragments does not preclude binding of the primary antibody to Fc-Receptors! Blocking with normal serum from the same host as the secondary may not be sufficient. If you encounter background caused by Fc-receptor binding, blocking with IgG or IgG-Fc-Fragments of the examined species may be helpful. In this case, a secondary antibody that is adsorbed against the examined material may additionally improve the results.

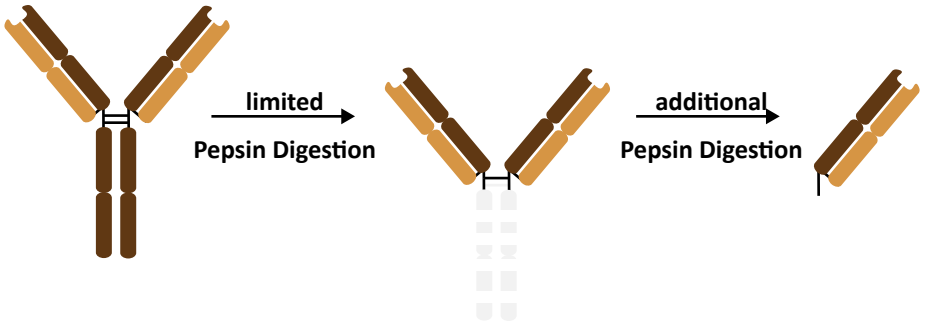


Figure: Proteolytic pretreatment of antibodies with Papain results in cleavage in two monovalent Fab-Fragments and one Fc-Fragment.

The proteolytc treatment with Pepsin results in a shortended Fc-Fragment and one bivalent F(ab')2-Fragment.

Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	**Format**	Species Reactivity	Specificity	Conjugation	Adsorbtion

Examples Fab-Fragment usage

Differentiate between two Primay Antibodies from the same Species

Rabbit Antibodies:

- Rabbit anti-Antigen X
- Rabbit anti-Antigen Y
- Rabbit IgG from non-immunized animals

Goat Antibodies:

- Goat anti-Rabbit IgG (H+L)
- Goat Fab-Fragment anti-Rabbit IgG (H+L)

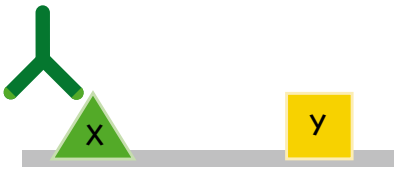
Mouse Antibodies:

- Mouse anti-Goat IgG (H+L) (adsorbed against Ms, Hu und Rb serum proteins)
- Mouse anti-Rabbit IgG (H+L) (adsorbed against Ms, Hu, Go und Sh serum proteins)

Dyes (Label):

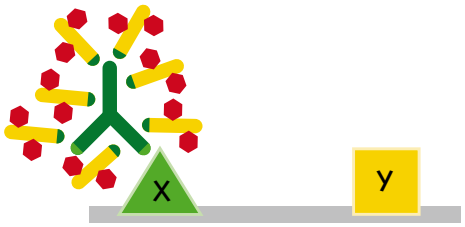
- Fluorescent Label 1 (green)
- Fluorescent Label 2 (red)

1



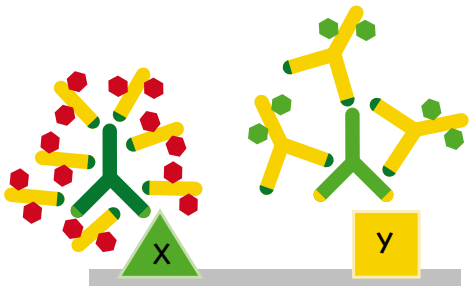
Incubation with the first primary antibody (anti X).

2



Incubation with an excess of Fab-Fragment antibodies conjugated to label 2, that is directed against the primary antibody.

3



Conventional detection of the second primary antibody (anti Y), using indirect immunofluorescence.

The method can be adapted by replacing the unconjugated antibody in step one by a conjugated antibody and blocking this antibody in step 2 with an unconjugated Fab-Fragment.

Note that a higher concentration of Fab-Fragments may be necessary to achieve a complete block of the first primary antibody. If this leads to an increase in background staining, a lower concentration of conjugated Fab-Fragments can be supplemented by unconjugated Fab-Fragments for additional blocking.

Attention: Aggregates of conjugated Fab-Fragmens can act as polyvalent molecules and bind the second primary antibody. This may lead to a (false positive) staining of the second antigen.

Examples Fab-Fragment usage

"Transforming" a Primary Antibody into a "Different Species"using Fab-Fragments

Rabbit Antibodies:

- Rabbit anti-Antigen X
- Rabbit anti-Antigen Y
- Rabbit IgG from non-immunized animals

Goat Antibodies:

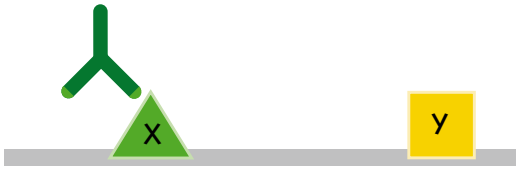
- Goat anti-Rabbit IgG (H+L)
- Goat Fab-Fragment anti-Rabbit IgG (H+L)

Mouse Antibodies:

- Mouse anti-Goat IgG (H+L) (adsorbed against Ms, Hu und Rb serum proteins)
- Mouse anti-Rabbit IgG (H+L) (adsorbed against Ms, Hu, Go und Sh serum proteins)

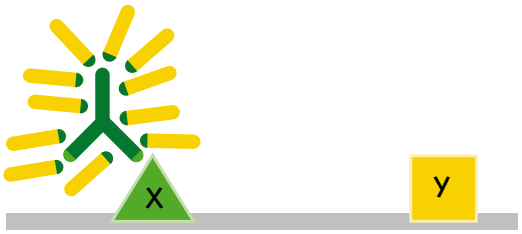
Dyes (Label): Fluorescent Label 1 (green) Fluorescent Label 2 (red)

1



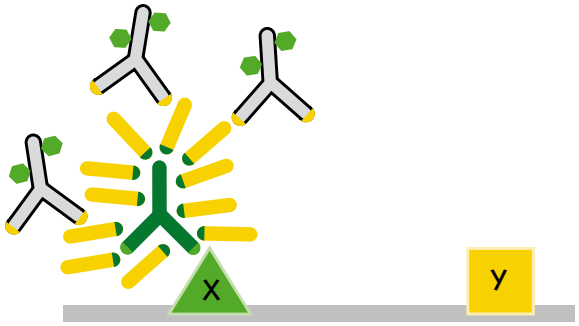
Incubation with the first primary antibody (anti X).

2



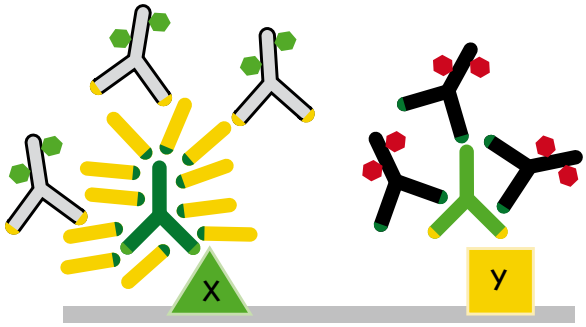
Incubation with an excess of unconjugated Fab-Fragment antibodies, that is directed against the primary antibody.

3



Incubation with a labelled tertiary antibody directed against the Fab-Fragment. It is important that the tertiary antibody does not react with any of the primary antibodies or the secondary antibody used to detect the anti-y primary antibody (see next step).

4 & 5



Incubation with a second primary antibody anti-Y and a corresponding conjugated secondary antibody. It is important that the secondary antibody does not react with the Fab-Fragment or the tertiary antibody used in step 3.

Due to the complexity of the setup, trying different concentrations of each reagent may have a positive impact on the staining results. Also, changing the order of the steps can lead to better results. In addition, blocking with corresponding normal serum in between steps may reduce background staining. To obtain satisfactory results some empirical trials may be necessary.

IgG (H+L)

Specificity

4. Step:

What should a Secondary Antibody detect?

In most immunological assays either polyclonal IgG antibodies (e.g. from from rabbit, goat, chicken etc.) or monoclonal antibodies from mouse, rat, hamster etc. that are isolated from B-cell hybridoma, are used. Secondary antibodies used as detection reagents should have a high sensitivity for their respective primary antibody target.

To manufacture a secondary antibody a whole IgG antibody is usually used as immunogen and the resulting antibody detects a multitude of epitopes that may be present on different classes or subclasses (see figure right).

In case only one primary antibody is used in an assay and no immunoglobulins from other species nor other classes or subclasses are present the specificity "IgG (H+L)" has the highest sensitivity and is usually the most suitable.

The selection is more complicated if more than one primary antibody (from different species) is used in the same experiment (multiple labeling). In this case cross-reactivity of secondary antibodies must be prevented. This is done by adsorbing the secondary against cross reactive antigens (Min X, see step 6).

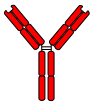
The optimal specificity for each of the secondary antibodies is still IgG (H+L)!

There are some exceptions to this rule that are explained in more detail on the next pages, in brief:

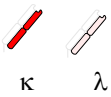
- Class specific antibody detection, while antibodies of other classes are present or simultaneous detection of different antibody classes.
- Detection of antibodies of certain subclasses / subclass determination
- Simultaneous detection of more than one subclass from one species.
- Quantitative equal detection of antibodies of different subclasses.
- Light Chain detection without unwanted detection of a Heavy Chain

Detection range of a mouse IgG (H+L) specific antibody

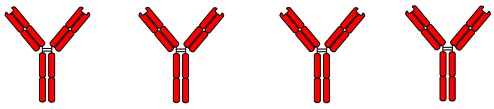
Immunogen: IgG(H+L)



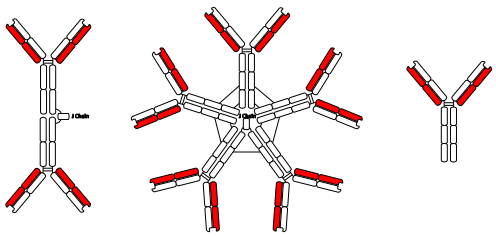
Detection:



Light Chain



IgG1 IgG2A IgG2B IgG3



IgA IgM IgE

An animal species (e.g. Goat) that is used to generate antiserum is immunized with an immunoglobulin of the target species (Mouse). Most commonly the immunoglobulins used for immunization are isolated from whole serum, that means that the mixture of subclasses corresponds to the normal distribution in serum.

Antiserum of animals immunized with IgG (H+L) comprises antibodies that are directed against the heavy chain and the light chain of IgG. This antiserum reacts with F(ab')2-fragments as well as with the Fc-Portion of the IgG antibody. This is the reason anti-IgG also cross-reacts well with all other immunoglobulin classes such as IgM, IgA, IgE as they share the same κ and λ light chains.

Anti IgG (H+L) antibodies that are not adsorbed (see page 15) react to a degree of 40% - 60% with light chains, wheras with highly adsorbed antibodies this reactivity is reduced to 9% - 30%.

Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	Format	Species Reactivity	**Specificity**	Conjugation	Adsorbtion

IgG (H+L)

Specificity - special cases

Step 4

Special considerations when choosing the specificity of a Secondary Antibody

Class specific antibody detection, while antibodies of other classes are present or simultaneous detection of different antibody classes

anti-IgG-Fc-Fragment

Immunizing an animal species (e.g. goat) with purified Fc-Fragment (e.g. from human or mouse) leads to the formation of antibodies that are highly specific for the heavy chain of IgG. As differences between Immunoglobulin classes are determined by the heavy chain, those antibodies do not recognize IgM, IgA and IgE.

In addition, Fc-Fragment specific antibodies are adsorbed against F(ab')2-Fragments and in some cases against IgM and IgG (human) or IgM (rat and mouse). Those Antibodies are marked as "Fcγ-specific" and have a cross-reactivity against IgM, IgA and light chains of less than 1% (ELISA).

anti-IgM (µ-chain), anti-IgM Fc5µ

To generate IgM-specific antibodies the host (e. g. goat) is immunized using purified IgM (mouse and rat) or trypsin-digested purified IgM Fc5µ (human).

After purification by affinity chromatography anti-Mouse and anti-Rat IgM µ-chain specific antibodies are adsorbed against IgG and anti-human IgM Fc5µ are adsorbed against IgG and IgA. Those IgM-specific antibodies have a cross reactivity against IgG or IgA of less than 1% (ELISA).

anti-IgA (α-chain) / anti-IgE (ε-chain)

Antibodies against IgA (α-chain) / IgE (ε -chain) are adsorbed against all other classes (i.a. IgG and IgM) so that no noteworthy cross-reactivity against other classes can be detected by ELISA.

Detection of antibodies of certain subclasses / subclass determination

Simultaneous detection of more than one subclass from one species

Subclass-specific anti-mouse antibodies against IgG1, IgG2a, IgG2b, IgG2c and IgG3

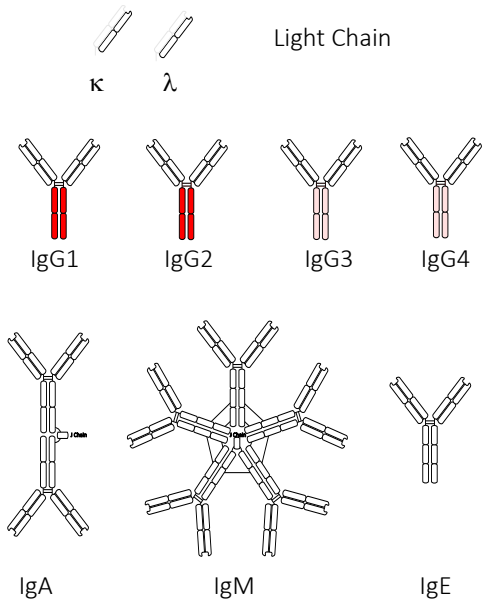
Due to their high specificity those antibodies should only be used if a subclass distingtion is required.

Anti-mouse subclass specific antibodies from Jackson ImmunoResearch are adsorbed and / or tested against reactivity to other sublasses, Fab-Fragments, IgG, IgM and Immunoglobulins from Human, Rabbit and Bovine.

Detection range of anti-human IgG Fc specific antibody

Immunogen: IgG(Fc)

Detection:



Anti-IgG (Fc) antibodies do not react equally well with all IgG-subclasses. The reason is the unequal distribution of subclasses in normal serum (which is used for immunization). IgG3 and IgG4 are not detected to the same extend as IgG1 and IgG2. In case IgG3 and / or IgG4 need to be detected anti IgG(H+L)orIgGF(ab')2-specificantibodiesshouldbeused.

IgG (H+L)

Spezificity - special cases

Step 4

Special considerations when choosing the specificity of a Secondary Antibody

Quantitative equal detection of antibodies of different subclasses.

anti-IgG F(ab')2-Fragment

These antibodies are made using IgG F(ab')2-Fragment as an immunogen. The heavy chain part of the IgG F(ab')2 causes a weak immune response and the resulting antibodies are - depending on their adsorbtion (see step 6) - directed to more than 60% against the light chain. In addition, anti F(ab')2-Fragment antibodies are adsorbed against IgG (Fc) and therefore react only with the Fab-part of IgG.

Anti-IgG F(ab')2-Fragment antibodies can be used to detect all IgG classes and subclasses (e.g. IgM, IgG1-4, IgA, etc.) to a similiar extend and can be used when different classes must be detected or when the subclass of the primary antibody is unknown.

anti-IgG + IgM (H+L) / anti-IgG + IgM + IgA (H+L)

In order to obtain a uniform reaction to the above classes, antibodies with these specificities are made by mixing antibodies against individual classes. In a mixture of anti-IgG + IgM (H+L) reactivity to light chains varies between 30% and 45%. The total reactivity to IgM (including light chain) is between 55%- 70%.

To detect mouse subclasses with equal sensitivity we recommend Goat anti-mouse IgG (Subclass 1, 2a, 2b and 3) Fcγ-Fragment-specific antibody (catalog-no. 115-XXX-164), that has a balanced reactivity to those subclasses.

Light Chain detection without unwanted detection of a Heavy Chain

Anti-Light chain specific antibodies

Anti-IgG light chain specific antibodies react with native primary antibodies that are used to detect proteins in Western blots.

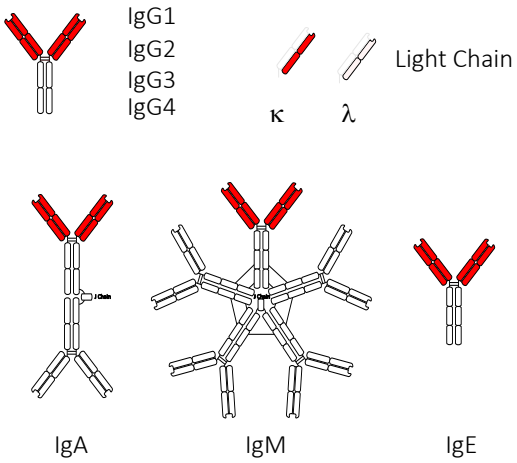
When using appropriate (!) dilutions they do not react with reduced or denatured heavy chains (50 kDa) of the IgG molecule, that might overlay targets at 50 kDa obtained by immunoprecipitation (IP) on blots.

Anti-light chain antibodies show a strong reactivity to **native** IgG light chains. Sensitivity to reduced / denatured light chains can be reduced. These products are not recommended for a sensitive / quantitative detection of light chains in Western blotting.

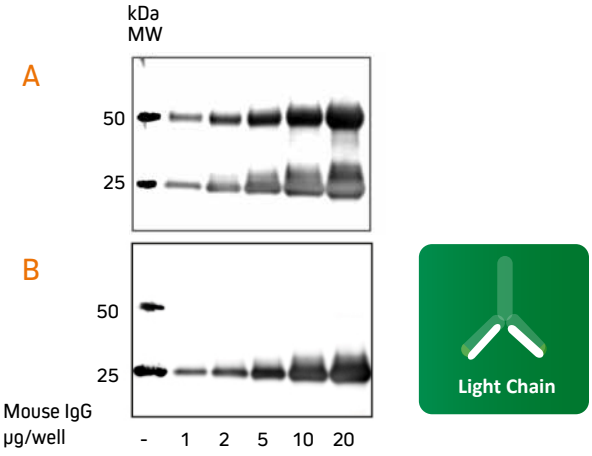
Detection range of anti-IgG F(ab')2-Fragment antibodies

Immunogen: F(ab')2-Fragment

Detection:



The resulting antibodies predominantly detect κ-light chains. Detection of primary antibodies with a λ-light chain is limited.



Detection of mouse IgG with anti-mouse IgG (H+L) (115-035-003) **(A)** and anti-mouse light-chain-specific antibody (115-035-174) **(B)**. The anti-light chain antibody allows detection of target proteins that are overlayed by the 50 KDa antibody band (Fig. Jackson ImmunoResearch).

Biotin

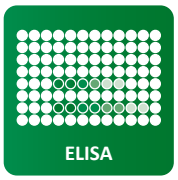
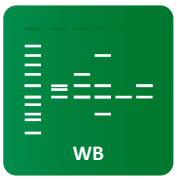
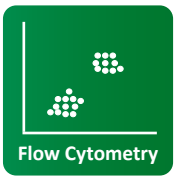
Conjugation

Step 5:

How to select the right Conjugate?

Selection of a conjugate depends on the intended assay the antibody will be used in and the method of detection (enzymatic, fluorecent, direct, indirect etc.). Especially before selecting a fluorecent dye it is important that the correct excitation sources and filters are available. For fluorescent based methods in immunohistochemistry and cytochemistry we recommend photostable dyes such as Carbocyanides (Cy2, Cy3, Cy5) or AlexaFluor®-Dyes instead of traditional dyes such as FITC or TRITC. Some of the available dyes are also recommended for super resolution microscopy. For STED Alexa Fluor 488, FITC und Alexa Fluor 594 are recommended and for STORM Alexa Fluor 488, FITC, Cy3, Alexa Fluor 647, Cy5 and Alexa Fluor 790 are suitable.

Reporter / Label for different applications and recommended dilutions

Conjugate	 ELISA	 WB	 IF/ICC	 IHC	 Flow Cytometry
unconjugated			10-20 µg/ml		
Cy™2, FITC			1:50 – 1:200	1:50 – 1:200	1:50 – 1:200
Cy™5			1:100 – 1:400	1:100 – 1:400	1:100 – 1:400
DyLight™-/Alexa®-Conjugates , Cy™3			1:100 – 1:800	1:100 – 1:800	1:100 – 1:800
Alexa®-680- / 790-conjugates			1:50.000 –1:200.000		
Phycoerythrin			1:50 – 1:200	1:50 – 1:200	1:50 – 1:200
Peroxidase (HRPO)	1:5.000 – 1:100.000	1:5.000 – 1:100.000 (non-ECL)	1:500 – 1: 5.000	1:500 – 1: 5.000	
		1:10.000 –1:200.000 (ECL)			
Alkaline Phosphatase	1:5.000 –1:50.000	1:5.000 –1:50.000	1:500 – 1:5.000		
Biotin for use with					
- Streptavidin-Fluorescent Label			1:200 – 1:1.000	1:200 – 1:1.000	1:200 – 1: .000
- Enzyme-conjugated Streptavidin	1:20.000 –1:400.000	1:20.000 –1:400.000	1:500 – 1:5.000	1:500 – 1:5.000	
4 nm Gold			1:20 – 1:200		
6, 12 nm Gold			1:20 – 1:40		
18 nm Gold			1:10 – 1:20		

Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	Format	Species Reactivity	Specificity	**Conjugation**	Adsorbtion

FITC

Conjugation

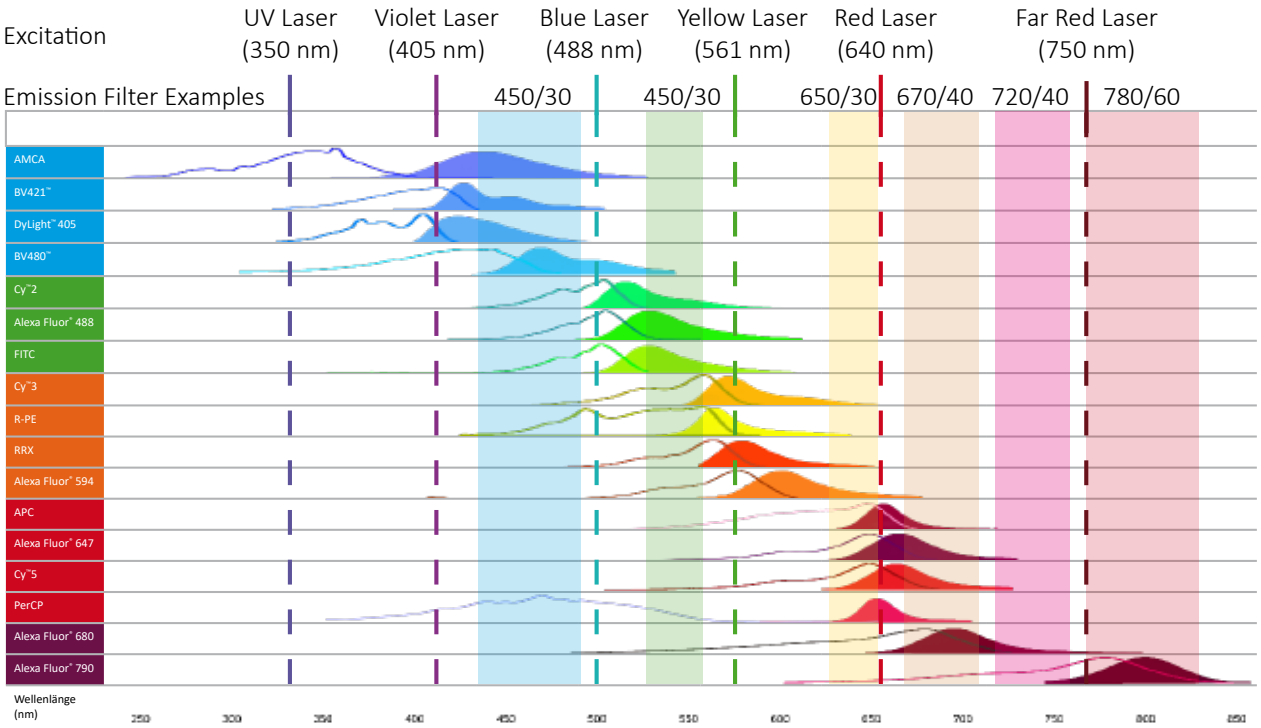
Step 5:

Fluorophores: Excitation and Emmision Maxima of Fluorescent Dyes

Enzyme Conjugates and Substrates

Selection of Fluorochromes for Immunofluorecent Stainings depend on:

1. availability of light sources , filter sets and detection system on your microscope
2. required degree of color separation in multiple labeling experiments: For example: Rhodamin Red-X, Alexa Fluor 594 or Alexa Fluor 647 have a better separation from Alexa Fluor 488 or FITC compared to Cy3.
3. abundance of antigen expression: antigens with a low level of expression should be detected with brighter fluorophores while antigens with higher expression can be detected using less bright fluorophores.
4. required sensitivity: generally Alexa Fluor 488, Cy3, Alexa Fluor 594 and Alexa Fluor 647 are brighter than FITC, Cy2, TRITC, Rhodamin-Red X, Texas Red and Cy5.



Overview of popular fluorecent dyes, excitation sources and emmision filters (Fig. by Jackson ImmunoResearch).

Enzymes and popular substrates:

Enzyme	Substrate	Color
Alkaline Phosphatase	BCIP/NBT	dark blue-violet
	Fast Red	red (intensive red)
	Neufuchsin	pink (fuchsin red)

Enzyme	Substrate	Color
HRPO	DAB	brown
	AEC	red brown
	TMB	dark blue

Our secondary antibody nomenclature helps you to find the right antibody!

Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh
Host Species	Format	Species Reactivity	Specificity	**Conjugation**	Adsorbtion

minX

Adsorbtion

Step 6:

Determining the necessary degree of (pre)-adsorption for the Secondary Antibody

Ig-specific antibodies against one species cross-react with immunoglobulins and serum proteins of any other species to a higher or lesser extend. To circumvent this problem antibodies are often adsorbed against other species. This step leads to antibodies that have a cross-reaction of less than 1 % for the adsorbed species. Adsorbed antibodies are recognizable by the addition "*minimal cross-reaction to (MinX)*".

Adsorbed *(MinX)* antibodies are recommended when immunoglobulins from other species may interfere with the results by cross-reactivity.

It should be noted that antibodies that are adsorbed against closely related species have only a reduced epitope recognition and may detect some monoclonals with limited sensitivity.

Anti-Rat and anti-Mouse Antibodies / tissue in the same assay

This is especially true for anti-mouse antibodies with minimum cross-reactivity against rat or anti-rat antibodies with minimum cross-reactivity against mouse but also for anti-hamster antibodies with minimum cross-reactivity against mouse and/or rat.

Antibodies with minimal cross-reactivity against closely related species may also recognize some IG-Subclasses (especially IgG2b, IgG2c and IgG3) to a lesser degree. This is due to the fact that some subclasses have larger homologies to the related species than others.

For example, anti-mouse IgG that has been adsorbed against rat should only be used when the primary from mouse is a) used on rat tissue that contains rat IgGs or b) on other tissues if a second primary from rat is also used.

Bovine serum albumin (BSA) and bovine milk powder often contain contaminating IgGs that can react with anti-bovine, anti-goat, anti-equine, anti-sheep antibodies.

If BSA or milk powder is used as a blocking reagent or diluent for these antibodies, it may lead to reduced antibody titers or high background staining. To solve this problem Jackson ImmunoResearch offers IgG-free BSA (cat no 001-000-161).

Detecting anti-Goat Antibodies

When detecting primary goat antibodies, we recommend bovine anti-goat antibodies (805-xxx-180), that do not have any cross reaction against bovine.

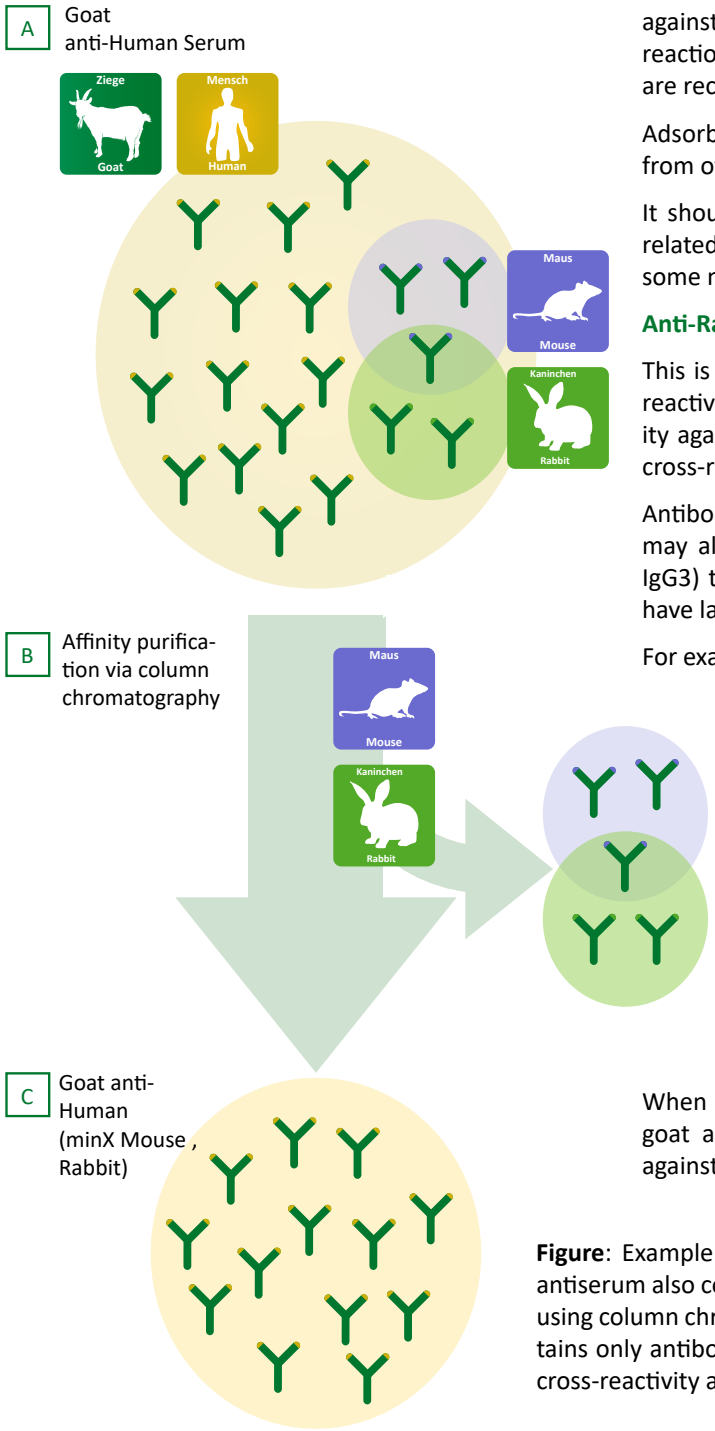


Figure: Example of an adsorbtion of anti-human antibodies from goat. **(A)** The antiserum also contains cross-reacting antibodies against mouse and rabbit. **(B)** By using column chromatography against mouse and rabbit the resulting fraction contains only antibodies against human. **(C)** The resulting antibodies have a minimal cross-reactivity against mouse and rabbit (MinX Ms, Rb).

Our secondary antibody nomenclature helps you to find the right antibody!

Host Species	Format	Species Reactivity	Specificity	Conjugation	Adsorbtion
Donkey	F(ab')2	anti-Mouse	IgG (H+L)	-Biotin	MinX Bo,Ck,Go,Gp,Hm,Ho,Hu,Rb,Sh

Online Sekondary Antibody Selection Guide

With more than 8,500 available products the selection of the right secondary reagent is not always easy. This selection guide will help you to make the right choices!

An easy way to find the right secondary is the search tool on our website. By making the respective selections you are guided to the antibodies that fit your needs!

If you still have questions you can of course contact our technical team by phone or e-mail to find the right antibody.

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