

Immunoglobulins

A GUIDE TO IMMUNOGLOBULINS STRUCTURE AND FUNCTION

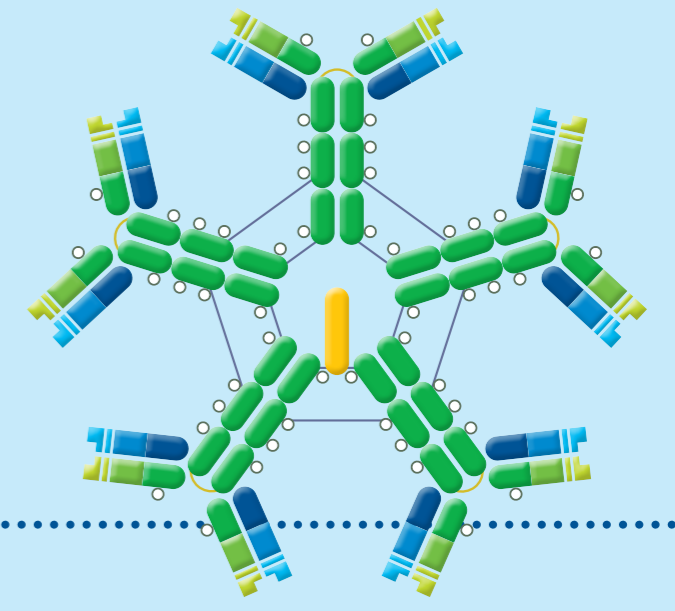
Structure and function of Immunoglobulins

Immunoglobulins form one branch of the adaptive immune system, recognizing pathogens or foreign material and initiating an immune response. Also known as antibodies, immunoglobulins are a group of glycoproteins present in the serum and tissue fluids of all mammals. Some on the surface of B cells act as antigen specific receptors, others circulate unbound in the blood and lymph. There are a number of immunoglobulin classes, which differ in size, charge, amino acid composition, and nature of glycosylation. Each immunoglobulin is bifunctional. The Fab region is responsible for antigen binding, and the Fc region for binding cellular receptors, conferring its effector function. The structure of all immunoglobulins consists of four chains: two

identical light chains and two identical heavy chains make up the recognizable Y shape of the antibody. The chains are held together by inter-chain disulfide bonds and by non-covalent interactions which vary between the different immunoglobulin isotypes. Intra-chain disulfide bonds within each of the polypeptide chains are responsible for the folded nature of the light and heavy chains. These make up the discrete regions called the variable and constant domains. Each light chain consists of a variable and a constant domain, VL and CL. The heavy chains have a variable domain (VH) and, depending on the molecule, may have 2 or more constant domains (CH). The exposed ends of the VL and VH domains are called complementarity determining regions

(CDRs), and are responsible for the molecule's ability to recognize antigenic epitopes. The junction between the 1st and 2nd CH domains is a flexible area called the hinge region, which allows the two antigen-binding sites to operate independently. The constant heavy chains below the hinge make up the Fc fragment responsible for antibody effector function. Carbohydrates are found at conserved locations on the CH domain of most immunoglobulins, where they influence protein folding, secretion, and molecular interactions. Hinge length and glycosylation, combined with the number of inter-chain disulfide bonds, contribute to structural differences which ultimately create the variety in biological function of the immunoglobulin classes.

Immunoglobulin isotypes



IgM
IgM exists in 2 forms. Monomeric IgM is found on B cells, functioning as a B cell receptor (BCR). Pentamers (hexamers have also been found) that consist of 5 identical antibody units are found in circulation. These pentamers confer a high overall avidity from 10 binding sites, making IgM a potent complement activating antibody. IgM has an extra domain on the mu chain (CH4) and contains a J chain which is essential for the active transport of IgM through epithelial cells (see IgA). IgM does not cross the placenta, but is the first Ig to be made by the fetus and the first Ig to be made after immunization or exposure to antigen. Elevated levels of IgM may indicate recent infection or exposure to antigen.

Antigen binding region (Fab)

Antigen binding takes place at sites on the Fab (Fragment antigen binding) fragment, located at the end of the variable domains. The most variable region of both heavy and light chains is the Hyper Variable or Complementarity Determining Region (HVR/CDR), which determines which epitope the Ig recognizes. Typically 3 hypervariable regions exist on both the VL and VH domains, containing the polypeptide sequences that interact with the antigen, and provide the vast diversity the immune system needs to recognize a multitude of antigens. Four framework regions (FR) between the CDRs stabilize their position.

Immunoglobulin classes and types

Immunoglobulin (Heavy chain) isotypes
The five immunoglobulin isotypes (classes) found in humans are named according to the heavy chains present in the molecule. An IgG molecule has heavy chains known as gamma (γ) chains, IgM has mu (μ) chains, IgA has alpha (α) chains, IgE has epsilon (ε) chains and IgD has delta (δ) chains.

Immunoglobulin (Light chain) isotypes
Immunoglobulin type is also classified by the light chains present. The light chains may be either kappa (κ), or one of 4 lambda (λ) subtypes, and either light chain can be associated with any of the heavy chains. The ratio between κ and λ light chains varies between species, e.g. humans' ratio of κ to λ light chains is about 60:40, whereas the ratio in mice is 90:10.

F(ab)₂ fragment

Pepsin digestion of the Ig molecule results in cleavage of the hinge region after the inter-chain disulfide bond. This results in a divalent fragment that contains both antigen binding sites called F(ab)₂. The F(ab)₂ fragment can bind antigen and crosslink, but it cannot mediate effector functions due to loss of the Fc fragment. The average molecular weight of a F(ab)₂ fragment is about 110 kDa.

Fragments yielded by immunoglobulin proteolysis

Proteolytic digestion of immunoglobulins cleaves the molecule into its component fragments.

Fab fragment

Digestion of the Ig molecule with pepsin cleaves the molecule at the hinge region before the inter-chain disulfide bond, resulting in cleavage of the Fc fragment from two identical monovalent Fab (fragment antigen binding) fragments. The Fab fragments contain the antigen binding site (CDR) of the antibody. Each Fab fragment is approximately 50 kDa and made up of the VL, CL, VH and CH1 domains.

Fc fragment - the crystallizable fragment

Pepsin digestion of the Ig molecule yields the Fc fragment - Fragment crystallizable. The Fc was the first domain of the Ig structure to be solved by crystallography, but only after proteolysis, highlighting the flexibility of the molecule conferred by the Ig hinge region. The Fc fragment, derived from constant domains of the heavy chains, determines the effector function of the immunoglobulin: binding to specific cell receptors and complement proteins, and mediating different physiological effects (cell lysis, degranulation of mast cells, basophils and eosinophils, and other processes).

IgG

IgG is important in the humoral immune response, providing protection from pathogens by neutralization, opsonization, and the complement pathway. IgG neutralizes viruses and bacteria by binding to pathogen surface proteins, preventing the pathogen from entering host cells and replicating. In opsonization, IgG binds pathogens to phagocytic cells (macrophages and neutrophils) via their Fc receptors (FcγR), mediating phagocytosis and pathogen destruction. A final mode of protection comes via the complement pathway, in which IgG-coated pathogen is recognized by C3q protein. Binding of C3q to multiple Ig on the pathogen induces its conformational change which activates the classical complement cascade and subsequent clearance of the immunoglobulin-pathogen complex by phagocytosis and lysis.

IgY

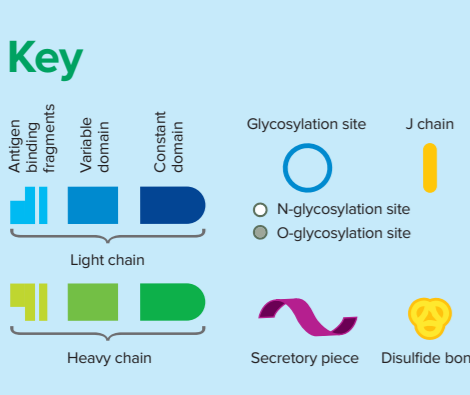
Immunoglobulin Y (IgY) is found in birds. IgY possesses a fourth CH domain and unique oligosaccharide side chains, but lacks a well-defined hinge region, making it inflexible. Similarities between IgY, IgE, and IgG suggest that IgY may be an ancestral molecule to both IgG and IgE. IgY plays a similar biological role to IgG, providing defense against infectious agents. It mediates anaphylactic reactions, a function limited to IgE in mammals. Structural differences with mammalian immunoglobulins are significant: IgY does not bind to mammalian Fc receptors or Protein A or G. It does not interact with mammalian complement proteins or rheumatoid factors, making IgY primary antibodies useful in diagnostic assays.

IgE

IgE is monomeric with an extra CH domain (CH4). IgE serum levels are low in healthy individuals; high IgE levels may indicate inflammatory disease. When IgE binds to Fc receptors on basophils and mast cells, the activated cells release their granules, histamine, heparin, leukotrienes, and other compounds eliciting immediate hypersensitivity (allergic) reactions. IgE does not fix complement nor is it agglutinating, but it recruits eosinophils to clear parasitic infection.

IgD

IgD exists as a monomer and is found in low levels in serum. The role of IgD is uncertain, although its expression may correlate with the elimination of autoreactive B cells. IgD is co-expressed with IgM on B cell surfaces where it functions as antigen-specific BCR. Presence of IgD serves as a marker for the differentiation of B cells into their mature form. IgD does not bind complement. IgD may bind to basophils and mast cells, activating these cells to produce antimicrobial factors that function in respiratory immune defense.



Fab and F(ab)₂ fragment antibody applications

Fab and F(ab)₂ antibodies can offer benefits over whole IgGs in certain applications. F(ab)₂ fragments can be used to avoid binding to endogenous Fc receptors or to Protein A or Protein G. Monovalent Fabs can be used to block endogenous immunoglobulins on cells, tissue or other surfaces. Fabs can also be used in multiple labeling experiments, allowing primary antibodies raised in the same species to be used. For more details on how Fab and F(ab)₂ antibodies can be used, please see our website.

	IgA		IgG			IgE	IgM	IgD
Isotype	α		γ			ε	μ	δ
Binding sites	2, 4		2			2	10	2
Structure	Monomer in serum, dimers in secretions		Monomer			Monomer	Pentamer	Monomer
Subclasses	IgA1	IgA2	IgG1	IgG2	IgG3	None	None	None
Total MW (kDa)	165 (Monomer) 400 (Dimer)	165 (Monomer)	150	150	170	150	190	970
Number of N-linked Glycosylation sites per chain	2	2	1	1	1	1	6	5
Distribution	Intravascular and secretions (milk, sweat, tears, seminal fluids)		Intra- and extravascular			Saliva and nasal secretions	Intravascular	Lymphocyte surface
Function	Protects mucous membranes - antibacterial with lysozyme		Secondary response antibody - antibacterial and antiviral			Allergic reactions and parasitic infections	Neonatal immunity and first line of humoral defense	Undefined

Table 1. Structural and functional characteristics of the human immunoglobulins.

Antibody specificity

Using the right antibody specificity can allow you to optimize your experiment. Jackson ImmunoResearch produces a number of affinity-purified secondary antibodies which recognize whole Ig molecules and specific Ig fragments. Considering antibody specificity during experimental design can improve assay performance and analysis.

Detect a broad range of epitopes with Anti-IgG (H+L) specificity
Anti-IgG (H+L) antibodies react with both the heavy and light chains of the IgG molecule, i.e. with both the Fc and Fab₂. Fab fragments of IgG. Anti-IgG (H+L) antibodies also react with other immunoglobulin classes (IgM, IgA, IgD, and IgE), and subclasses since they all share the same light chains (either kappa or lambda). Anti-IgG (H+L) antibodies have broader epitope recognition than anti-fragment specific antibodies. They are suggested for all general immunodetection procedures.

Detect the Fc fragment with Anti-IgG, Fc/Fc₂ fragment specific antibodies
Anti-IgG, Fc fragment specific antibodies react with the Fc fragment of IgG. They have been tested by ELISA and/or adsorbed against Fab fragments. In some cases (anti-human, anti-mouse and anti-rat) they are additionally tested and/or adsorbed to minimize cross-reactivity to IgM and/or IgA, and are labeled "Anti-IgG, Fc₂". These antibodies are commonly used to detect IgG without recognizing the other immunoglobulin classes.

Detect IgM with Fc_μ fragment/μ chain specificity
Antibodies described as Fc_μ or μ chain specific recognize the IgM heavy chains but not the light chains common to all whole Igs, and therefore can be used to bind IgM without recognition of other immunoglobulin classes. They have been tested by ELISA and/or adsorbed against IgG, and Fc_μ specific is also tested against IgA.

Distinguish mouse IgG subclasses with Anti-Mouse IgG, Fcγ subclass specificity
Subclass-specific antibodies allow for discrimination among the Fc fragments of the 5 mouse subclasses: IgG1, IgG2a, IgG2b, IgG2c and IgG3. Anti-Mouse IgG, Fcγ subclass specific antibodies are intended for distinguishing between different subclasses of mouse IgG primary antibodies in multiple labeling experiments. They can also be used for isotyping mouse monoclonal antibodies. They have been tested by ELISA and/or adsorbed to minimize cross-reactivity to other subclasses, Fab fragments, IgM, and a few other species of IgG.

Avoid detecting heavy chains with Anti-IgG, Light Chain specific antibodies
Anti-IgG, Light chain specific antibodies can be used to avoid detection of the reduced and denatured IgG heavy chains of the immunoprecipitating (IP) antibody when Western blotting after immunoprecipitation, revealing bands from proteins of interest in the 50 kDa range.

Recognize all immunoglobulin classes with Anti-IgG, F(ab)₂ fragment specificity
These antibodies react with the F(ab)₂ / Fab region of IgG. They have been tested by ELISA and/or adsorbed against Fc fragments. They are not specific for IgG since they react with light chains, and therefore also react with other immunoglobulin classes (IgA, IgM, IgD, and IgE).

Be more specific with minimally cross-reactive secondary antibodies (min X... Sr Prot)
Secondary antibodies raised against one species may cross-react with other species and cause unwanted signal unless they have been specifically adsorbed against the other species. Antibodies with "min X... Sr Prot" in the description have been tested and/or adsorbed against IgG and/or serum proteins of those species indicated in the parentheses. Choose min X... Sr Prot secondary antibodies when the presence of immunoglobulins from other species may lead to interfering cross-reactivities. When considering antibodies adsorbed against closely-related species (for example mouse and rat), be aware that the antibodies have greatly reduced epitope recognition and may recognize some monoclonals poorly.

See our website for application notes.

IgA

Serum IgA is monomeric, whereas dimeric secretory IgA is found in all mucosal secretions. Dimer formation is facilitated by Fc domain "tails", and an interconnecting peptide (J chain), which facilitates transport of IgA across the epithelial mucosa (transcytosis). Dimeric IgA binds with polymeric immunoglobulin receptor (pIgR) present on the epithelial cells. The complex is then internalized and transported via vesicles through the cytoplasm of the cell. Proteolytic cleavage of the pIgR releases the antibody to the epithelial mucosa. The extracellular domain of the pIgR is retained by the secreted dimeric IgA as the secretory component. IgA acts as a neutralizing antibody and is important in mucosal immunity, preventing invading pathogens attaching and penetrating epithelial surfaces. IgA has two subclasses in humans.