

# ANTIBODIES

## ISOTYPE FAMILIES



## Switch natural isotypes

- ❖ Clinically relevant monoclonal antibodies
- ❖ Fine-tuned effector functions
- ❖ Up to 14 different native and engineered isotypes

InvivoGen provides a series of clinically relevant antibodies in their original format or with different immunoglobulin isotypes. Our engineered antibodies are designed to adjust their effector functions, including half-life, complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP). The variety of the immunoglobulin constant regions helps you determine the most suitable isotype for your application.

## Antibodies against various targets

Anti-hCD20	Anti-hPD1
Anti-hCTLA4	Anti-hPD-L1
Anti-hEGFR	Anti-hTNF- $\alpha$
Anti-HER2	Anti-hVEGF

[WWW.INVIVOGEN.COM/ANTIBODY-ISOTYPES](http://WWW.INVIVOGEN.COM/ANTIBODY-ISOTYPES)

## Description

Monoclonal antibodies (mAbs) have become a major tool in the treatment of cancer and auto-immune diseases. The efficacy of antibodies is governed by their **bifunctional nature**. On the one hand, the variable domain of the immunoglobulin, within the fragment of antigen binding (Fab), confers **antigen specificity function**. On the other hand, the fragment crystallizable region (Fc) in the constant domain of the immunoglobulin triggers **antibody-mediated effector functions** by engaging a variety of Fc receptors. Antibody isotype switching is a biological process enabling changes in the ability of the antibody to interact with different Fc receptors and thus, reduce or potentiate effector functions. InvivoGen's antibody **isotype families** consist of **clinically relevant mAbs** comprising the same variable domain and the constant domain of various isotypes, therefore differing in their suitability for a given application.

## Native and engineered isotypes

### • Native isotype antibodies

Physiological native isotypes trigger various combinations of effector functions that are summarized in the table below.

### • IgG1NQ isotype antibodies

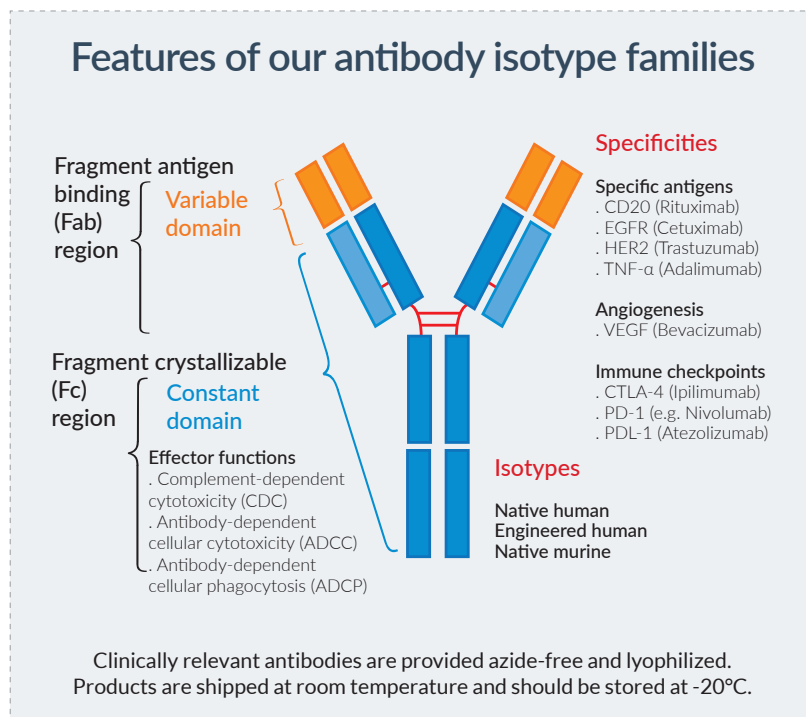
This isotype contains a N-glycosylation mutation of the constant region where potential asparagine (N) glycosylation sites are substituted by glutamine (Q) residues. These mAbs are non-glycosylated and their effector mechanisms mediated through the Fc receptor (FcγRI, FcγRII and FcγRIII), and the C1q component of the complement, are severely compromised or ablated.

### • IgG1fut isotype antibodies

The constant region of these mAbs is not fucosylated. This results in dramatic enhancement of ADCC without any change in CDC.

### • IgG4 (S228P) isotype antibodies

IgG4 antibodies undergo a process known as Fab arm exchange that potentially reduces their therapeutic efficacy. IgG4 (S228P) mAbs contain an engineered hinge region mutation (S228P) designed to prevent exchange of IgG4 molecules.



	Native human isotypes								Engineered human isotypes			Native murine isotypes		
Effector functions	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgE	IgG1NQ	IgG1fut	IgG4 (S228P)	IgG1	IgG2a	IgA
ADCC	++	+/-	++	+/-	+	+	+	+	-	++++	+/-	+/-	++	+
ADCP	+++	+/-	++	+	-	+	+	-	-	+++	+	+	+++	+
CDC	++	+	+++	-	+++	-	-	-	+/-	++	-	-	++	-

## Antibody specificities

### • Human CD20

CD20 (also known as B1) is a cell-surface marker expressed in both malignant and normal B cells. CD20 plays a role in the differentiation process of B cells into plasma cells. The hCD20 antibody family features the variable region of **rituximab**, a chimeric human/mouse IgG1 mAb. Binding of rituximab to CD20 results in cell destruction through different mechanisms including direct signaling of apoptosis, complement activation and cell-mediated cytotoxicity.

### • Human CTLA4

CTLA-4 (also known as CD152) is transiently expressed by activated T cells and highly expressed by regulatory T cells and it transduces negative intracellular signals upon binding to CD80 and CD86. The hCTLA4 antibody family features the variable region of **ipilimumab** which is a fully human IgG1 mAb. Ipilimumab blocks the immunosuppressive action of CTLA-4 and enhances antitumor T-cell responses. In addition, ipilimumab induces ADCC and TNF- $\alpha$  production.

### • Human EGFR

The epidermal growth factor receptor (EGFR) is over-expressed in cancer cells and plays a key role in their proliferation and survival. The anti-hEGFR family features the variable region of **cetuximab**. Cetuximab is a chimeric human/mouse IgG1 mAb that targets EGFR, a cell surface receptor over-expressed in many types of cancer. Binding of cetuximab to EGFR blocks ligand/receptor binding, and induces receptor internalization and subsequent degradation.

- **HER2 receptor**

The human epidermal receptor HER2 (or HER2/neu, or ERBB2) is expressed at the cell membrane of epithelial cells and plays an important role in normal cell growth and differentiation. However, in some cancers, particularly in breast and ovarian cancers, HER2 is over-expressed and causes uncontrollable cell proliferation. The HER2 antibody family features the variable region of **trastuzumab**. Trastuzumab is a humanized IgG1 mAb whose binding to HER2 results in cell death through ADCC and ADPC.

- **Human PD-1**

The programmed cell death 1 (PD-1) receptor is expressed mostly by activated T cells, but also by activated B cells, dendritic cells, monocytes and natural killer cells. Upon its binding to PD-L1, PD-1 mediates a negative intracellular signaling. The anti-hPD1 family features the variable region of **nivolumab** or **pembrolizumab**. Nivolumab is a fully human IgG4 (S228P) and pembrolizumab is a humanized IgG4 (S228P). These two mAbs bind and block the activation of PD-1, thus relieving immuno-suppression.

- **Human PD-L1**

The programmed cell death ligand 1 (PD-L1) is a transmembrane protein over-expressed on tumor cells and tumor infiltrating immune cells, such as macrophages. Binding of PD-L1 to PD-1 on cytotoxic T cells inhibits the antitumor immune response. The anti-hPD-L1 isotype family features the variable region of **atezolizumab**, a fully humanized IgG1 mAb that contains an Asp to Ala change introduced at position 298 (N298A) to eliminate its ability to bind to human Fcγ receptors. Atezolizumab is a blocking mAb targeting both human and mouse PD-L1.

For studies in mice, InvivoGen has developed anti-hPD-L1-mIgG1, containing a mouse IgG1 Fc domain to prevent anti-drug-antibodies. Anti-hPD-L1-mIgG1 InvivoFit™ is available at a preclinical grade, sterile and endotoxin-free (<1 EU/mg).

- **Human TNF-α**

The tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine mainly secreted by macrophages and is implicated in a number of diseases, notably autoimmune diseases and cancer. The TNF-α antibody family features the variable region of **adalimumab**, a fully human mAb which blocks the interaction of TNF-α with TNF receptors, thereby downregulating the inflammatory reactions associated with autoimmune diseases.

- **Human VEGF**

The vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis and it is over-expressed in many tumors. The anti-hVEGF family features the variable region of **bevacizumab**, a humanized IgG1 mAb which blocks VEGF interaction with the VEGF receptor thus inhibiting downstream pathways that regulate cell growth and angiogenesis.

## Antibody Isotype Families

PRODUCT	ISOTYPE	QUANTITY*	CAT. CODE
<b>Anti-hCD20 antibodies, variable region of rituximab</b>			
Anti-hCD20-hIgG1**	Human IgG1	100 µg	hcd20-mab1
Anti-hCD20-hIgG1NQ	Human IgG1, non-glycosylated	100 µg	hcd20-mab12
Anti-hCD20-hIgG1fut	Human IgG1, non-fucosylated	100 µg	hcd20-mab13
Anti-hCD20-hIgG2**	Human IgG2	100 µg	hcd20-mab2
Anti-hCD20-hIgG3	Human IgG3	100 µg	hcd20-mab3
Anti-hCD20-hIgG4	Human IgG4	100 µg	hcd20-mab4
Anti-hCD20-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hcd20-mab14
Anti-hCD20-hIgM	Human IgM	100 µg	hcd20-mab5
Anti-hCD20-hIgA1**	Human IgA1	100 µg	hcd20-mab6
Anti-hCD20-hIgA2**	Human IgA2	100 µg	hcd20-mab7
Anti-hCD20-hIgE	Human IgE	100 µg	hcd20-mab8
<b>Anti-hCTLA4 antibodies, variable region of ipilimumab</b>			
Anti-hCTLA4-hIgG1	Human IgG1	100 µg	hctla4-mab1
Anti-hCTLA4-hIgG1NQ	Human IgG1, non-glycosylated	100 µg	hctla4-mab12
Anti-hCTLA4-hIgG1fut	Human IgG1, non-fucosylated	100 µg	hctla4-mab13
Anti-hCTLA4-hIgG2	Human IgG2	100 µg	hctla4-mab2
Anti-hCTLA4-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hctla4-mab14
Anti-hCTLA4-hIgA2	Human IgA2	100 µg	hctla4-mab7
<b>Anti-hEGFR antibodies, variable region of cetuximab</b>			
Anti-hEGFR-hIgG1	Human IgG1	100 µg	hegfr-mab1
Anti-hEGFR-hIgG1NQ	Human IgG1, non-glycosylated	100 µg	hegfr-mab12
Anti-hEGFR-hIgG1fut	Human IgG1, non-fucosylated	100 µg	hegfr-mab13
Anti-hEGFR-hIgG2	Human IgG2	100 µg	hegfr-mab2
Anti-hEGFR-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hegfr-mab14
Anti-hEGFR-hIgA2	Human IgA2	100 µg	hegfr-mab7

\* Larger quantities are available upon request \*\* Murine isotypes available, please visit our website: [www.invivogen.com/anti-hcd20](http://www.invivogen.com/anti-hcd20)

PRODUCT	ISOTYPE	QUANTITY*	CAT. CODE
<b>Anti-hHER2 antibodies, variable region of trastuzumab</b>			
Anti-HER2-Tra-hIgG1	Human IgG1	100 µg	her2tra-mab1
Anti-HER2-Tra-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	her2tra-mab14
Anti-HER2-Tra-hIgA2	Human IgA2	100 µg	her2tra-mab7
<b>Anti-hPD1 antibodies, variable region of nivolumab or pembrolizumab</b>			
Anti-hPD1-Ni-hIgG1	Human IgG1	100 µg	hpd1ni-mab1
Anti-hPD1-Ni-hIgG1NQ	Human IgG1, non-glycosylated	100 µg	hpd1ni-mab12
Anti-hPD1-Ni-hIgG1fut	Human IgG1, non-fucosylated	100 µg	hpd1ni-mab13
Anti-hPD1-Ni-hIgG2	Human IgG2	100 µg	hpd1ni-mab2
Anti-hPD1-Ni-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hpd1ni-mab114
Anti-hPD1-Ni-hIgA2	Human IgA2	100 µg	hpd1ni-mab7
Anti-hPD1-Pem-hIgG1	Human IgG1	100 µg	hpd1pe-mab1
Anti-hPD1-Pem-hIgG1NQ	Human IgG1, non-glycosylated	100 µg	hpd1pe-mab12
Anti-hPD1-Pem-hIgG2	Human IgG2	100 µg	hpd1pe-mab2
Anti-hPD1-Pem-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hpd1pe-mab14
Anti-hPD1-Pem-hIgA2	Human IgA2	100 µg	hpd1pe-mab7
<b>Anti-hPD-L1 antibodies, variable region of atezolizumab</b>			
Anti-hPD-L1-hIgG1	Human IgG1	100 µg	hpd1l-mab1
Anti-hPD-L1-hIgG1 (N298A)	Human IgG1, (N298A)	100 µg	hpd1l-mab12
Anti-hPD-L1-hIgG1fut	Human IgG1, non-fucosylated	100 µg	hpd1l-mab13
Anti-hPD-L1-hIgG2	Human IgG2	100 µg	hpd1l-mab2
Anti-hPD-L1-mIgG1	Murine IgG1	100 µg	hpd1l-mab9
Anti-hPD-L1-mIgG1 InvivoFit™ (preclinical grade)	Murine IgG1	5 x 1 mg	hpd1l-mab9-5
<b>Anti-hTNF-α antibodies, variable region of adalimumab</b>			
Anti-TNF-α-hIgG1***	Human IgG1	100 µg	htnfa-mab1
Anti-TNF-α-hIgG2***	Human IgG2	100 µg	htnfa-mab2
Anti-TNF-α-hIgG3	Human IgG3	100 µg	htnfa-mab3
Anti-TNF-α-hIgG4	Human IgG4	100 µg	htnfa-mab4
Anti-TNF-α-hIgM	Human IgM	100 µg	htnfa-mab5
Anti-TNF-α-hIgA1***	Human IgA1	100 µg	htnfa-mab6
Anti-TNF-α-hIgA2***	Human IgA2	100 µg	htnfa-mab7
Anti-TNF-α-hIgE	Human IgE	100 µg	htnfa-mab8
<b>Anti-VEGF, variable region of bevacizumab</b>			
Anti-hVEGF-hIgG1	Human IgG1	100 µg	hvegf-mab1
Anti-hVEGF-hIgG4 (S228P)	Human IgG4 (S228P)	100 µg	hvegf-mab14
Anti-hVEGF-hIgA2	Human IgA2	100 µg	hvegf-mab7

\*Larger quantities are available upon request \*\*\* Murine isotypes available, please visit our website: [www.invivogen.com/anti-htnfa](http://www.invivogen.com/anti-htnfa)

## Antibody isotype controls

As negative controls, InvivoGen provides several human, murine and rat antibody isotype controls targeting *E. coli* β-galactosidase.

All antibody isotype controls are generated by recombinant DNA technology, produced in CHO cells and purified by affinity chromatography.

Human isotype controls	hIgA2, hIgG1, hIgG1 (N298A), hIgG2, hIgG3, hIgG4, hIgG4 (S228P)
Murine isotype controls	mIgG1, mIgG2a, mIgG2b
Rat isotype controls	rIgG1, rIgG2a, rIgG2b