

In vivo Isotype Control Antibodies

High concentration, low endotoxin, no preservatives, bulk amounts

CrownVivo™ isotype antibodies are the leading source for *in vivo* isotype controls among researchers in discovery, preclinical and clinical areas.

CrownVivo™ antibodies are functional grade isotype controls* manufactured specifically for *in vivo* animal studies including antigen neutralization and pathway/blockade depletion assays during development and validation of antibody biotherapeutics. These isotype controls are designed to avoid cross-reactivity with common preclinical species and supplied in the exact format needed for *in vivo*, *ex vivo*, or *in vitro* studies.

*CrownVivo™ isotype control antibodies are manufactured by Crown Biosciences, Inc., a JSR Life Sciences Company.

- **Monoclonal antibodies**
- **Endotoxin: <1 EU/mg as determined by LAL method**
- **Purity: >95% by SDS Page and SEC-HPLC***
- **Concentration: >4mg/mL**
- **Buffer: Aseptically packaged in PBS (40mM KH₂PO₄, 150mM NaCl, pH 7.4) with no carrier protein or preservatives added.**

*(>95% by SDS Page, >90% by SEC-HPLC for Product C0005)

Strict LOT to LOT conformity and reliability

PRODUCT CODE	TARGET	PRODUCT NAME
C0001	Human IgG1 kappa	In vivo Grade Human IgG1 kappa Isotype Control – CrownVivo™
C0002	Human IgG2 kappa	In vivo Grade Human IgG2 kappa Isotype Control – CrownVivo™
C0003	Human IgG3 kappa	In vivo Grade Human IgG3 kappa Isotype Control – CrownVivo™
C0004	Human IgG4 kappa	In vivo Grade Human IgG4 kappa Isotype Control – CrownVivo™
C0005	Mouse IgG1 kappa	In vivo Grade Mouse IgG1 kappa Isotype Control – CrownVivo™
C0006	Mouse IgG2a kappa	In vivo Grade Mouse IgG2a kappa Isotype Control – CrownVivo™
C0008	Mouse IgG2b kappa	In vivo Grade Mouse IgG2b kappa Isotype Control – CrownVivo™
C0010	Rat IgG1 kappa	In vivo Grade Rat IgG1 kappa Isotype Control – CrownVivo™
C0020	Human IgG1 (D265a) kappa	In vivo Grade Human IgG1 (D265a) kappa Isotype Control – CrownVivo™
C0045	Human IgG4 kappa (S228P)	In vivo Grade Human IgG4 kappa (S228P) Isotype Control – CrownVivo™

For Research Use Only. Not for use in diagnostic procedures.

What are Isotype Control Antibodies?

Isotype control antibodies are negative controls used to accurately measure antibody drug effects and efficacy for *in vitro* and *in vivo* monoclonal antibody (mAb) studies. Isotype controls match the test/primary antibody characteristics, but are raised against antigens not found in common preclinical species; this means that isotype controls lack specificity for the target antigen. In both *in vivo* and *in vitro* assays, a signal can result from various binding events. In order to accurately interpret assay results, it is critical to differentiate between binding events such as:

- mAb binding in an antigen-dependent specific manner.
- mAb binding in a non-antigen dependent manner, due to interaction with Fc receptors (FcR) or other proteins.

Isotype control antibodies provide an ideal negative control for mAb efficacy studies, enabling researchers to accurately distinguish between non-specific background and specific antibody signal.

How are Isotype Controls Important to Drug Development?

Isotype Controls Mimic Widespread FcR Engagement Effects

Isotype control antibodies are needed in *in vivo* efficacy assays to mimic the widespread FcR engagement that occurs via the Fc region of the test antibody. This engagement can induce effects which will only be properly analyzed when compared with an isotype control.

For example, when testing mAb *in vivo* efficacy in immunoncology research, different degrees of tumor growth inhibition (TGI) can be calculated depending on if an isotype control or PBS is used as a negative control. Therefore, comparison of the test antibody needs to be against an isotype control to make sure the antibody effect is not over- or underestimated.

Changes in tumor infiltrating lymphocyte (TIL) populations can also be observed when comparing negative controls (isotype control v. PBS). The extent of these changes varies by which model is used, making anticipation of the effect difficult during study design. Using an appropriate control is important for immunoprofiling and analysis of downstream results.

Isotype Controls Mimic FcR and Protein Staining for Flow Cytometry

In flow cytometry experiments, test antibodies can cause non-specific staining by binding to FcRs or other proteins. As FcR expression varies across tissue type, this non-specific staining can appear highly specific, confounding the results of your study. A matched isotype control should mimic this non-specific binding and staining, allowing more precise analysis than if PBS (which won't bind) is used.

Matching isotype control antibodies for flow cytometry experiments can be technically tricky. All isotype control comparator molecules should be fully characterized before use to avoid producing misleading results. The negative controls should match:

- Species
- Heavy chain
- Light chain
- Fluorophore conjugation as required

With this level of complexity, there is often some debate over isotype controls being used in flow cytometry experiments.

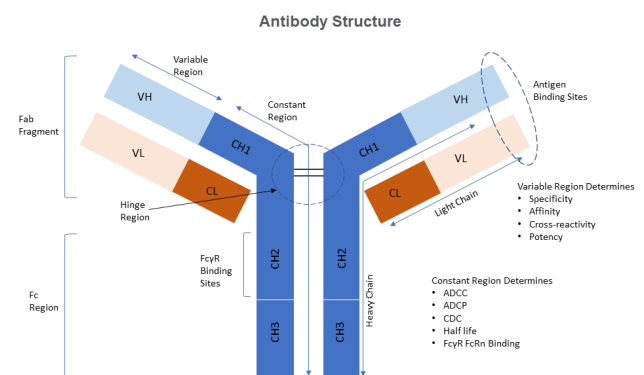
Isotype Controls are Important Negative Controls for Immunohistochemistry Experiments

Alongside *in vivo* studies, negative controls are also needed for assays such as immunohistochemistry (IHC). Isotype controls are the perfect negative control, provided that primary antibody concentration and isotype are matched.

Direct secondary antibody staining, or staining with a polyclonal pool from the same species as the primary, are sometimes used as negative controls. However, neither is likely to match the primary antibody isotype or replicate primary antibody binding patterns in the tissue as well as an isotype control can.

If you stain with a secondary only, you run the risk of the antibody binding directly to the slide section rather than binding to the primary/isotype control. A secondary antibody alone would not be complexed to a primary antibody, so it could have altered antigen-independent binding properties. The secondary antibody isotype could also cause an obvious difference in staining due to interacting with resident FcRs (which vary across tissue types).

Staining with a polyclonal pool as a negative control brings a separate roster of issues. This introduces a mixture of immunoglobulins which may bind to varied and additional targets, increasing background and non-specific staining events.



The constant regions help mediate antibody mechanism of action by coordinating binding to the Fc receptors (FcRs). FcRs are expressed on a large number of different cell types in the immune system and interact with an antibody differently based on antibody isotype. This makes certain isotypes better as drug candidates than others.

Contact us today for special formulation or bulk requests!

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