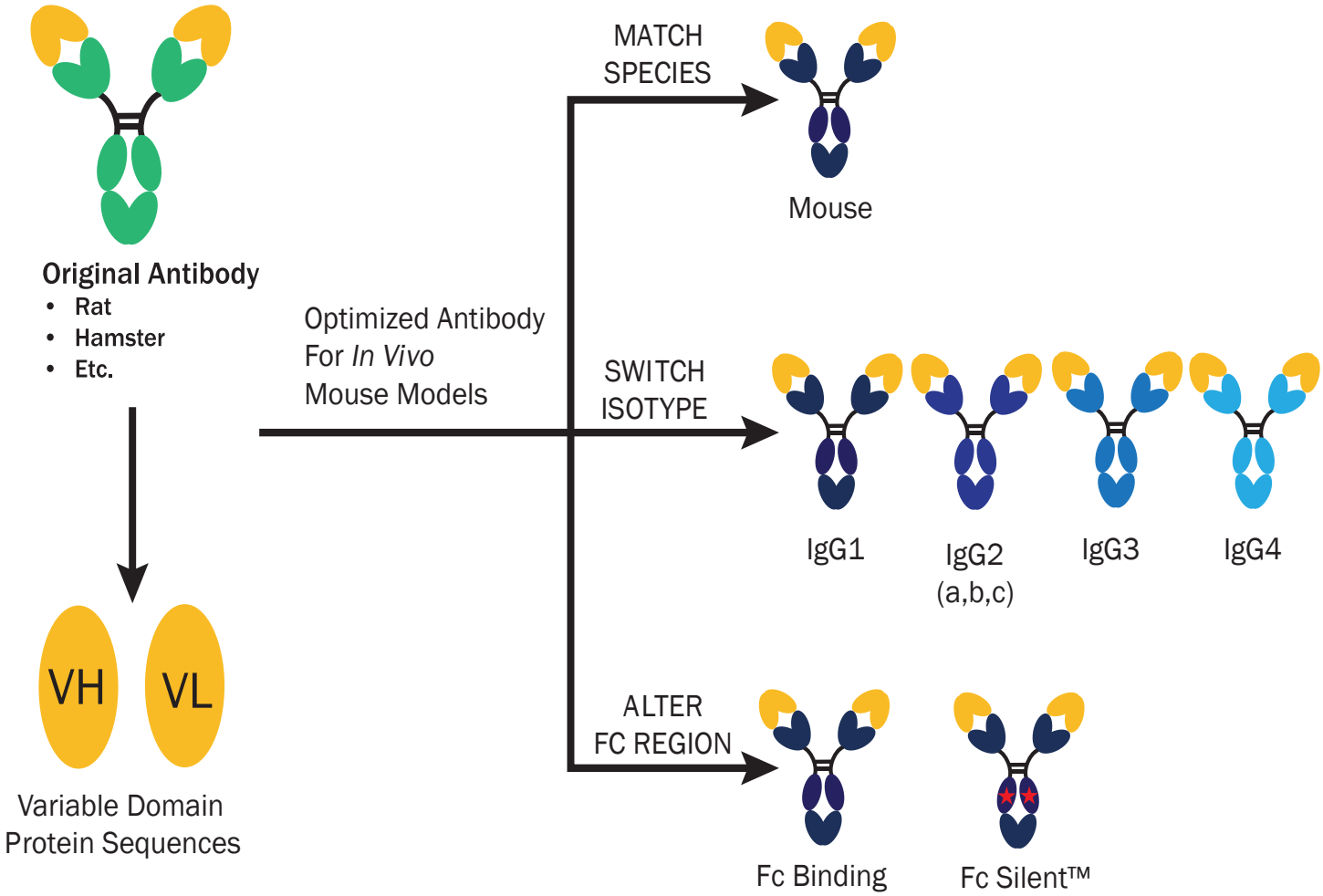
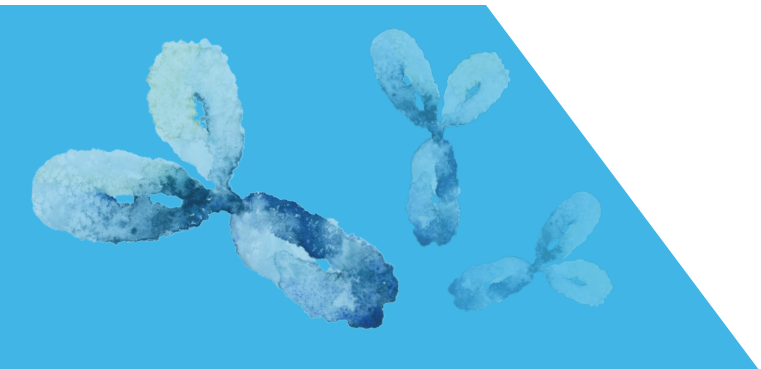
A Question of Isotype: How Switching Antibody Isotypes and Fc Regions Promotes Discovery

White Paper





absolute antibody
an Absolute Biotech Company

When it comes to in vivo experiments in mice, not all antibodies are equally suited to the task. Many of the most popular antibody clones for cancer research in murine models are derived from rats or hamsters, but these species can elicit detrimental immune responses in mice treated over time. Additionally, new research continues to show that Fc region binding affinity differs between isotypes and subtypes, resulting in variable antibody function and therapeutic effects. It is therefore critical to take into account antibody species, IgG subtype, and Fc silencing when selecting antibodies for in vivo research.

FcRs and Antibody Effector Function

Antibodies have many mechanisms of action. They can prevent or diminish a physiological function by binding to a ligand or receptor and blocking activation of the signaling pathway. The resulting diminished function can result in the loss of a cell's activity, lowered cell proliferation, blockade of immune-suppressing signals, apoptosis, or sensitization of a cell to cytotoxic agents (1).

For such actions, Fc-dependent effector functions are often not required and can indeed lead to mixed mechanisms of actions, confounding clear readout of the effects of target protein blockade and neutralization. Polesso et al. (2) found that mixing effector functions can unintentionally deplete antigen specific cells, reducing therapeutic efficacy of antibody treatments in tumor models. Moreno-Vicente et al. (3) further suggest that the optimal PD-1 blockade for immune checkpoint inhibition involves anti-PD-1 antibodies with non-binding Fc regions.

Our study on the effect of syngeneic mouse anti-PD-1 antibody efficacy capitalized on these aforementioned results, finding that the most effective anti-tumor effects were seen in mice treated with species-matched antibodies with Fc Silent™ mutations to prevent confounding Fc-dependent effector functions (4) (Figure 1).

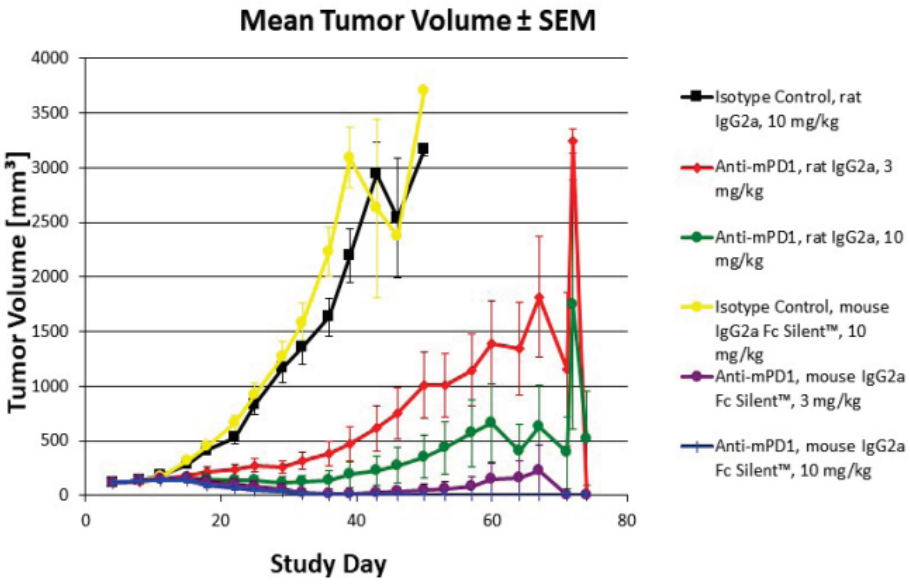


Figure 1. Mean tumor sizes in animals treated with PD-1 mouse IgG2a Fc Silent™ antibody based on clone RMP1-14 (Ab00813-2.3) are significantly smaller than in animals treated with the traditional rat IgG2a antibody (Ab00813-7.1) at a variety of concentrations.

Cell-Depleting Activity Commonly Achieved by Fc-Dependent Antibody Effector Functions

Antibodies bound to antigens on a target cell can activate the classical compliment pathway (also known as complement dependent cytotoxicity, or CDC) by interacting with the C1 complex, causing a complex to form that attacks the target cell.

Antibodies can also recruit immune-effector cells to trigger antibody-dependent cellular cytotoxicity (ADCC), a cell-destroying process that requires the cooperation of cellular and humoral players in the immune system. To activate this process, the variable binding domain of the antibody binds to its target and immune-effector cells bind to the Fc domain then lyse the target cell.

Fc receptors (FcRs) are present on immune-effector cells like natural killer cells and macrophages. They are what the Fc region of antibodies bind to in ADCC and CDC, but not all mouse IgG subtypes are equal in their Fc receptor binding affinity and fine-specificity, and thus their effector function potency. The table below outlines the relative specificities for activating and inhibitory Fc receptors, including Fc gamma receptors (FcγRs) and neonatal Fc receptors (FcRns).

Relative Mouse IgG Subtype Binding Affinity

Mouse IgG Receptor	High Affinity	Moderate Affinity	Low Affinity	No Binding
FcγRI	IgG2a, IgG2b		IgG3	IgG1
FcγRII		IgG1, IgG2a, IgG2b		IgG3
FcγRIII		IgG1, IgG2a, IgG2b		IgG4
FcγRIV	IgG2a, IgG2b			IgG1, IgG3
FcRn	IgG1, IgG2a, IgG2b, IgG3			

Figure 2. Table based on data from Bruhns (2012) (5) and Stewart et al. (2014) (6). Classification differs between studies and we recommend consulting the references for more in-depth information that would be beyond the scope of this piece.

Many antibodies that activate signaling pathways do so by mimicking ligand binding through receptor cross-linking. This can be efficiently mediated by antibodies cross-linked via their Fc domain on FcγR expressing cells. While antibodies with high FcγR binding ability most readily cross-link on cells that express FcγR, a balance with ADCC-mediated depletion of the target cell has to be struck.

This balancing act can be achieved by using mouse IgG1 antibodies, which can be cross-linked by inhibitory Fc receptors without inducing significant lytic effector functions. However, in some cases the use of a more potent FcγR engager such as IgG2a brings additional benefits as illustrated below on the examples of OX40 and TIGIT. Our VivopureX™ line of engineered recombinant antibodies has multiple subtypes available and nonbinding Fc region options for most targets to provide researchers with the right tools to explore full antibody therapy potential. Figure 3 (see page 4) illustrates how VivopureX™ antibodies are species swapped and reformatted into more clinically relevant versions.

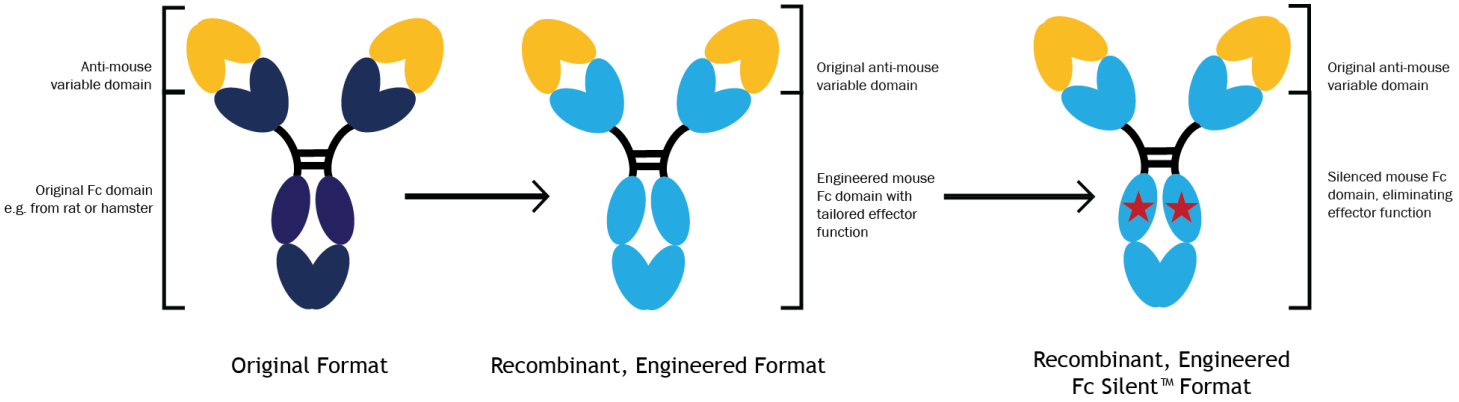


Figure 3. How VivopureX™ antibodies are engineered from their original species and format to recombinant, engineered mouse formats, including options for tailored or eliminated effector function.

Unlocking Research with Engineered Antibodies

Here are some of the ways anti-mouse antibodies have been altered with Fc region species and isotype matching for improved suitability for the specific research question at hand.

CTLA-4

CTLA-4, a receptor found on T cells responsible for abrogating T cell function, is a target in two of the current FDA-approved antibody therapies (7). Anti-CTLA-4 antibodies' therapeutic effects have been understood to be caused by the recruitment of effector T cells that result when you block CTLA-4, but new data suggest there is more to the anti-tumor story.

Selby et al. show how conversion of the original mouse IgG2b subtype to a mouse IgG2a subtype greatly increases anti-tumor activity of the 9D9 anti-CTLA-4 antibody clone in both MC38 (C57BL/6 mice) and CT26 (BALB/c mice) models (8). This mechanism involves both a blockade of CTLA-4 as well as depletion of tumor-infiltrating regulatory T-cells, resulting in lower inhibition and more effective anti-tumor performance in model murine immune systems. Additionally, Fc region alterations affected anti-tumor activity in the mice. Antibodies with a mutation that eliminates FcγR binding were shown to have reduced anti-tumor activity, as they did not deplete tumor-infiltrating regulatory T-cells at the tumor site.

This study suggests that anti-CTLA-4 antibodies also promote anti-tumor activity by selectively reducing regulatory T cell counts in tumor sites, and it brings to light the importance of having the right tools to explore all facets of anti-CTLA-4 antibody treatment in live mouse models. Without the ability to switch the subtype of the anti-CTLA-4 antibody, as well as alter effector function, the full view of the 9D9 clone's anti-cancer potential would not be available. Our catalog features recombinant engineered formats of the anti-CTLA-4 antibody, available in a variety of isotypes and subtypes, and species-matched to optimize your *in vivo* mouse experiments.

OX40

The anti-tumor performance of antibodies directed at OX40, a co-stimulatory molecule expressed on the surface of activated T cells, can also be affected by swapping species and isotype. A 2016 study from Metzger et al. found that anti-mouse OX40 antibody was shown to have superior anti-tumor activity when reformatted from the original rat IgG1 into mouse formats (9). Conversion to a mouse IgG2a, a subtype known to have higher binding affinity for Fc gamma receptors, further improved anti-tumor activity by increasing cross-linking through strong FcγR engagement. We offer the anti-OX40/CD134 antibody in mouse IgG1 and mouse IgG2a formats to explore full cross-linking potential.

PD-1

PD-1, or programmed cell death protein, is a protein expressed on some T cells that can inhibit anti-cancer immune responses. A variety of promising antibody therapies are aimed at blocking this protein or its associated ligand, PDL-1. Anti-PD-1 antibody performance also depends on isotype and Fc region functionality. In mouse models, we compared the *in vivo* efficacy of the anti-PD-1 antibody with the original rat IgG2a subtype and our recombinant engineered mouse IgG2a subtype with abrogated Fc region binding (Fc Silent™ technology). Our species-matched engineered mouse IgG2a format with silenced Fc region function worked better and for longer than the rat format at shrinking tumors in mice (4) (Figure 4).

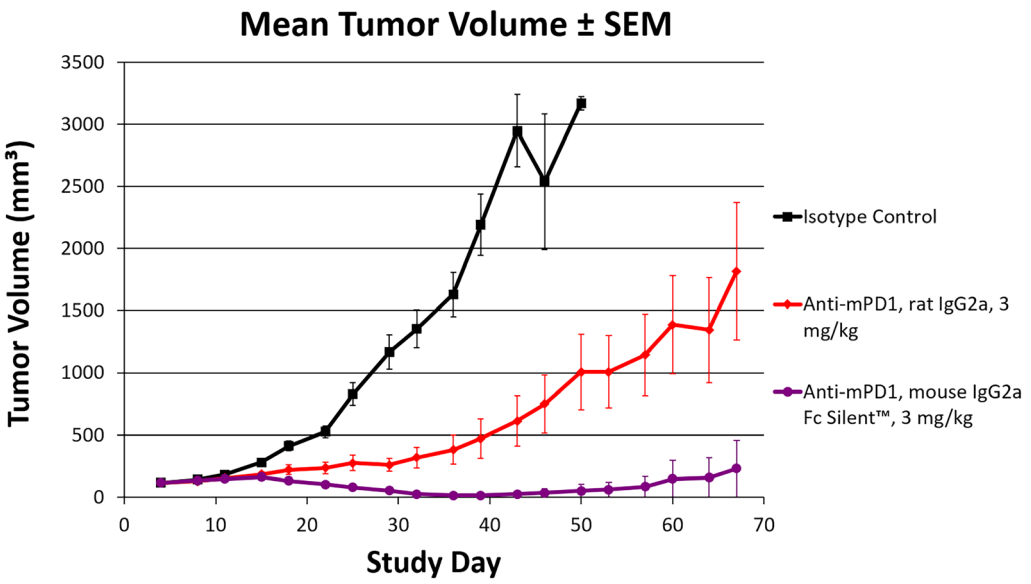


Figure 4. Mean tumor sizes in animals treated with PD-1 mouse IgG2a Fc Silent™ antibody based on clone RMP1-14 (Ab00813-2.3) are significantly smaller than in animals treated with the traditional rat IgG2a antibody (Ab00813-7.1).

In addition, Danahan et al. illustrate that to elicit the optimum PD-1 blockade and maximize anti-tumor activity, the anti-PD-1 antibodies used in treatment should have no Fc binding, as activating FcγRs reduces the efficacy of anti-PD-1 by eliminating CD8 tumor-infiltrating lymphocytes. Meanwhile, optimal anti-PDL-1 treatment requires FcγR binding to create a PD-1 blockade, so an Fc competent IgG subtype (known to have higher binding affinity for FcγRs) of anti-PDL-1 elicits the best anti-tumor response (10).

This evidence guides how we engineer the antibodies in our VivopureX™ antibody line. However, anti-PD-1 use in mouse models is not without its setbacks. Polesso et al. found that, sometimes, anti-PD-1 antibody therapies aimed at increasing the number of antigen-specific CD8 T cells results in the decrease of these T cells (2). The resulting unintentional target cell depletion is an issue with many common anti-PD-1 clones used in mouse models. Fortunately, our VivopureX™ catalog features Fc-silenced anti-PD-1 clones to mitigate target cell directed cytotoxicity and phagocytosis. Additionally, our custom services open up a wide range of antibody engineering to further address this phenomenon and others that inconvenience researchers using mouse models.

Anti-drug antibodies can lead to drops in therapeutic effectiveness even after a couple of injections.

Does your study account for antibody efficacy two weeks from now?

GITR

Glucocorticoid-induced TNFR-related protein (GITR) is another target for cancer immunotherapy and the most promising antibody treatments in preclinical experiments involve agonistic effects with GITR. However, prior to bringing this potential to the clinical stage, proper surrogate molecules can make the *in vivo* experimental process a lot more efficient. According to Belmar et al. (2017), Fc isotype matching of the anti-GITR antibody clone DTA-1 from its original rat IgG2b format to a mouse IgG2c format reduced anaphylaxis and anti-drug antibody generation in CB57/BL6 mice, while also improving anti-tumor efficacy (11). Our VivopureX™ line includes an anti-GITR antibody in mouse IgG2a to address the need for the right tools for *in vivo* mouse research.

CD25

The widely used anti-mouse CD25 antibody clone PC-61 in its original rat IgG1 lambda subtype is a poor depleter of mouse tumor-infiltrating regulatory T cells (Tregs), and therefore a poor surrogate for human anti-CD25 therapies which aim to reduce immune-suppressing Tregs. A 2017 study from Vargas et al. found that by engineering the CD25 antibody into a mouse IgG2a subtype, the antibody treatment is capable of Treg depletion (12). Additionally, when used in tandem with anti-PD-1 antibodies, the treatment eradicated established tumors.

This study further illustrates that switching the isotype of an antibody revived its clinical potential and created a molecule that can be used as a surrogate for mono- and combination immunotherapy research. For cell-depleting applications, IgG2a's and IgG2b's high affinities for activating Fc receptors make them the appropriate surrogate formats for many therapeutic antibodies (13), which is why we offer our anti-CD25 antibody in mouse IgG2a, among other formats.

Ly6G

The Ly6G (lymphocyte antigen 6 complex locus G6D) protein is expressed on myeloid-derived cells and can be used as a marker for neutrophils, the most abundant immune cell. It is capable of both pro- and anti-tumor activity (14); thus, more research is needed to realize its full implications in carcinogenesis and better tools to study this pathway in live mouse models are needed. In similar fashion to the anti-CD25 antibody described above, the anti-Ly6G antibody is a poor depleter of neutrophils.

However, a 2022 study published in Cells by Olofsen et al. showed that our VivopureX™ anti-mouse Ly6G antibody was able to deplete neutrophils in mice more efficiently than the original rat Ly6G-directed antibody (14) (15). Again, engineering antibodies to match the model species reduces adverse immune responses, and tailoring effector function can more efficiently result in desired effects like depleting target cells. Specifically, switching the isotype and tailoring effector function resulted in a better antibody tool to study neutrophils' role in cancer progression.

TIGIT

TIGIT (T cell immunoreceptor with Ig and ITIM domains) research is becoming increasingly better funded (16), opening new opportunities to harness yet another immune checkpoint inhibitor-directed antibody therapy for cancer treatment. New research shows that anti-TIGIT antibody performance in attacking tumors in live mouse models depends on its isotype. Han et al. illustrate that switching the mouse anti-TIGIT antibody from an IgG1 subtype to the IgG2a version increases the antibody's anti-tumor potency (17). This does not appear to be dependent on the increased depleting potential of IgG2a over IgG1, but rather on reverse activating signals triggered by IgG2a's high affinity for FcγRIV on myeloid cells, which can mediate antibody-dependent cellular cytotoxicity. We take isotype into account when curating our anti-TIGIT antibodies and recently added anti-TIGIT in a mouse IgG2a subtype to our catalog in response to this research.

A Question No Longer

As antibody experts, we appreciate the high specificity of antibodies to help us answer our questions. But are we properly utilizing the breadth of antibody options beyond just matching antibody and antigen? The above examples demonstrate that there are more variables in antibody performance than was previously understood—and even more variables for *in vivo* research.

One of the top considerations you should make is how antibody isotype contributes to your specific goal. Our VivopureX™ collection of recombinant engineered antibodies optimized for *in vivo* mouse models makes this decision easier. The antibodies in this line provide low immunogenicity in live mice with their species-swapped recombinant formats and industry-leading purity levels (<0.5 EU/mg, versus the <1 EU/mg or even <2 EU/mg standard for many reagent antibodies). Additionally, the formats we offer are informed by up-to-date literature to ensure that you have the most clinically relevant research reagents available to you.

Our custom antibody services open even more experimental possibilities. Our proprietary cloning system enables us to reformat antibodies into exactly what you need, and our antibody engineers are devoted to working with you directly to ensure your research needs are met. We can create chimerized and humanized antibodies, switch isotypes, manufacture fragments, and craft bispecific and multispecific antibodies. We can then express your custom antibodies on the milligram-to-gram scale, royalty-free, using our mammalian transient expression system. Absolute Antibody is devoted to bringing the right antibody reagents to the right researchers. Let our antibody expertise move your discoveries forward more efficiently.

References

1. Suzuki et al. (2015). Journal of Toxicologic Pathology, 28(3), 133–139.
2. Polesso et al. (2021). European Journal of Immunology, 51(6), 1473–1481.
3. Moreno-Vicente et al. (2022). Journal for ImmunoTherapy of Cancer, 10(1), e003735.
4. *Consistent, long-term PD1 blockade using a syngeneic, engineered anti-PD1 antibody*. Absolute Antibody.
5. Bruhns, P. (2012). Blood, 119(24), 5640–5649.
6. Stewart et al. (2014). Journal for ImmunoTherapy of Cancer, 2(1).
7. *Antibody therapeutics approved or in regulatory review in the EU or US.* The Antibody Society.
8. Selby et al. (2013). Cancer Immunology Research, 1(1), 32–42.
9. Metzger et al. (2016). Cancer Research, 76(13), 3684–3689.
10. Dahan et al. (2015). Cancer Cell, 28(3), 285–295.
11. Belmar et al. (2017). Journal of Immunology, 198(11), 4502–4512.
12. Vargas et al. (2017). Immunity, 46(4), 577–586.
13. Sionov, R. V. (2021). Cells, 10(9), 2486.
14. Olofsen PA et al. (2022). Cells, 11(21):3406.
15. *Engineered Mouse-Ly-6G Antibody Shows Near Complete Neutrophil Depletion In Vivo*. Absolute Antibody.
16. Dolgin, E. (2020). Nature Biotechnology, 38(9), 1007–1009.
17. Han et al. (2020). Frontiers in Immunology, 11.

About Us

Absolute Antibody

The Absolute Antibody vision is to make recombinant antibody technology accessible to all researchers. We offer antibody sequencing, engineering, and recombinant production as royalty-free custom services, as well as a unique reagents catalog of recombinant antibodies and Fc Fusion proteins, engineered into new and useful formats.

Absolute Biotech

Absolute Antibody is part of Absolute Biotech, a new company that unites multiple life science brands into one organization specializing in antibody reagents and services. Our mission is to serve as “antibody curators” for customers worldwide, treating each antibody like a work of art to deliver unique and absolutely defined reagents that empower scientists.

Learn more at absolutebiotech.com

Absolute Antibody, Ltd

absoluteantibody.com
info@absoluteantibody.com

UK Headquarters
Wilton Centre
Redcar TS10 4RF
+44 (0) 1642 688810

US Office
21 Drydock Avenue
Boston, MA 02210
+1 (617) 377-4057

absolute antibody
an Absolute Biotech Company