Unlocking Reliable and Cost-Effective Flow Cytometry with Anti-Mouse CD16/CD32 Antibodies

Flow cytometry is a cornerstone technique in immunology, enabling precise identification and quantification of cell populations based on protein markers. However, one common concern is the issue of nonspecific background staining caused by Fc receptor (FcR) binding. Bio X Cell's anti-mouse CD16/CD32 antibodies offer a robust solution, helping you reduce false positives, deliver reliable and reproducible results, even compatible with *in vivo* functional assays – all and saving on reagent costs.

Why block FcRs in flow cytometry?

Antibodies are Y-shaped molecules composed of two domains: a highly variable Fab (fragment antigen-binding) domain, which is responsible for recognizing and binding antigen, and an Fc (fragment crystallizable) domain, which facilitates the immune effector functions by binding to Fc receptors (FcR), such as antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), antigen presentation, and release of chemokine and cytokine [1,2].

However, FcR-Fc binding introduces false positive signals in cell analyses based on antigen-antibody reactions, such as flow cytometry assays. Pre-incubating samples with an Fc receptor blocker prevents such interference.

Expression and affinity of FcγRs

The constant region determines the antibody isotype, and Fc receptors are categorized according to the isotype they bind. For example, IgG, the most broadly used isotype in analytic experiments and drug development, has an Fc receptor called FcγR. Three subclasses of FcγR exist in humans and mice, including FcγRI (CD64), a high-affinity antibody that binds monomeric IgG, FcγRII (CD32) and FcγRIII (CD16), low-affinity antibodies that mainly bind to antigen-antibody immune-complexed (IC) IgG. In addition, mice have a unique high-affinity FcγRIV (CD16.2) [2].

FcγRs are found on most immune cells, especially myeloid cells. In mice, macrophages and monocytes highly express almost all FcγRs, dendritic cells also express several FcγRs, neutrophils express FcγRIV (CD16.2) and FcγRIII (CD16), B cells only express FcγRII (CD32), and NK cells solely express FcγRIII (CD16) [2,3].

When to block FcRs?

FcR blocking is a must when measuring cells expressing high levels of FcRs, such as monocytes and macrophages. Andersen et al. investigated FcR binding in flow assays in detail, and found that human peripheral blood mononuclear cells and monocyte-derived macrophages show strong nonspecific binding of mouse IgG1 and IgG2a, and such

binding can be eliminated by blocking either with a commercial FcR blocker, with mouse or human serum, or with mouse or human IgG in high concentration [4].

If the cell types of interest are not immune cells, such as tumor cells, it is still essential to prevent interference of tumor-infiltrating leukocytes for possibly immune cell enriched samples, like dissociated cells from solid tumors. In general, primary cells from solid tissues need to do FcR blocking in flow assays. In addition, FcR blocking significantly reduces noise and increases sensitivity for low expressing markers or rare cell types.

However, flow experiments do not always require FcR blocking. Cell lines or highly purified cells known to not express FcRs, such as tumor cells, epithelial cells, and T cells, do not have the risk of FcR-Fc binding. And recombinant antibodies with mutated Fc region eliminating FcR binding help to avoid such false positive signals from the very beginning.

Why choose Bio X Cell's anti-mouse CD16/CD32?

We can learn from the above study that a variety of reagents block FcRs. Among them, most commercial FcR blockers are purified antibodies to FcRs, for example, most mouse FcR blockers are anti-mouse CD16/CD32 antibodies, which has higher batch-to-batch consistency than serum and higher specificity than all IgG mixtures. Here we recommend Bio X Cell's anti-mouse CD16/CD32 antibodies for below strengths:

- **Well characterized:** Full disclosure of component, clone and concentration information. *InVivo*MAb anti-mouse CD16/CD32 (#BE0307) clone 2.4G2, isotype rat IgG2b, reacts specifically with the shared epitopes of mouse CD16 and CD32, and has also been reported to non-specifically bind FcγRI. Please kindly note that if a secondary antibody is required, do not use an anti-rat IgG2b antibody.
- **Fully validated:** Isolation and characterization of clone 2.4G2 was first reported in 1979 [5]. Now it is one of the most popular mouse FcR blocker clones. According to CiteAb, #BE0307 has empowered over 280 studies, covering various applications such as flow, immunofluorescence, magnetic bead sorting and functional experiments.
- **Premium Quality:** #BE0307 is an *InVivo*MAb product, with high purity (>95%) and low endotoxin (<2EU/mg), which is especially suitable for downstream functional assays, and can even be used for *in vivo* blocking. Bio X Cell also offers additional *InVivo*Plus version (#BP0307) with endotoxin below 1EU/mg.
- **Cost-effective:** Bio X Cell's anti-mouse CD16/CD32 antibodies are highly cost-effective based on the actual antibody content of the same clone.

Highlighted user case: Boosting anti-PD-1 therapy research

To investigate why sometimes tumors do not respond to PD-1 monoclonal antibody therapy, Arlauckas *et al* [6] used *in vivo* imaging to track the fate and activity of PD-1 antibodies in the tumor microenvironment and found that the antibody drug effectively binds to tumor-infiltrating PD-1$^+$ CD8$^+$ T cells within minutes of drug administration but is then captured by tumor-associated macrophages (TAMs), limiting the sustained effect of the drug. By blocking FcγRII/III in both *in vitro* co-cultures and *in vivo mouse* models with anti-mouse CD16/CD32, researchers showed that the transfer of PD-1 antibodies depends on the interaction between the drug's Fc domain and TAM's FcγRs:

o *In vitro* co-culture: fluorescently labeled PD-1 antibody was pre-incubated with T cells and then co-cultured with bone marrow-derived macrophages. The addition of antibody blocking FcγRII/III to the system significantly reduced the transfer of PD-1 antibody fluorescence from T cells to macrophages.

o *In vivo* mouse model: blocking FcγRII/III on peripheral macrophages by intraperitoneal injection of 200 μg of anti-mouse CD16/CD32 daily for 5 days prior to PD-1 antibody administration significantly prolonged the occupancy time of PD-1 antibody on tumor-infiltrating CD8$^+$ T cells and completely eliminated nonresponders, and the combination treatment in all mice achieving tumor rejection.

Explore Bio X Cell's anti-mouse CD16/CD32

*InVivo*MAb anti-mouse CD16/CD32

*InVivo*Plus anti-mouse CD16/CD32

Reference

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