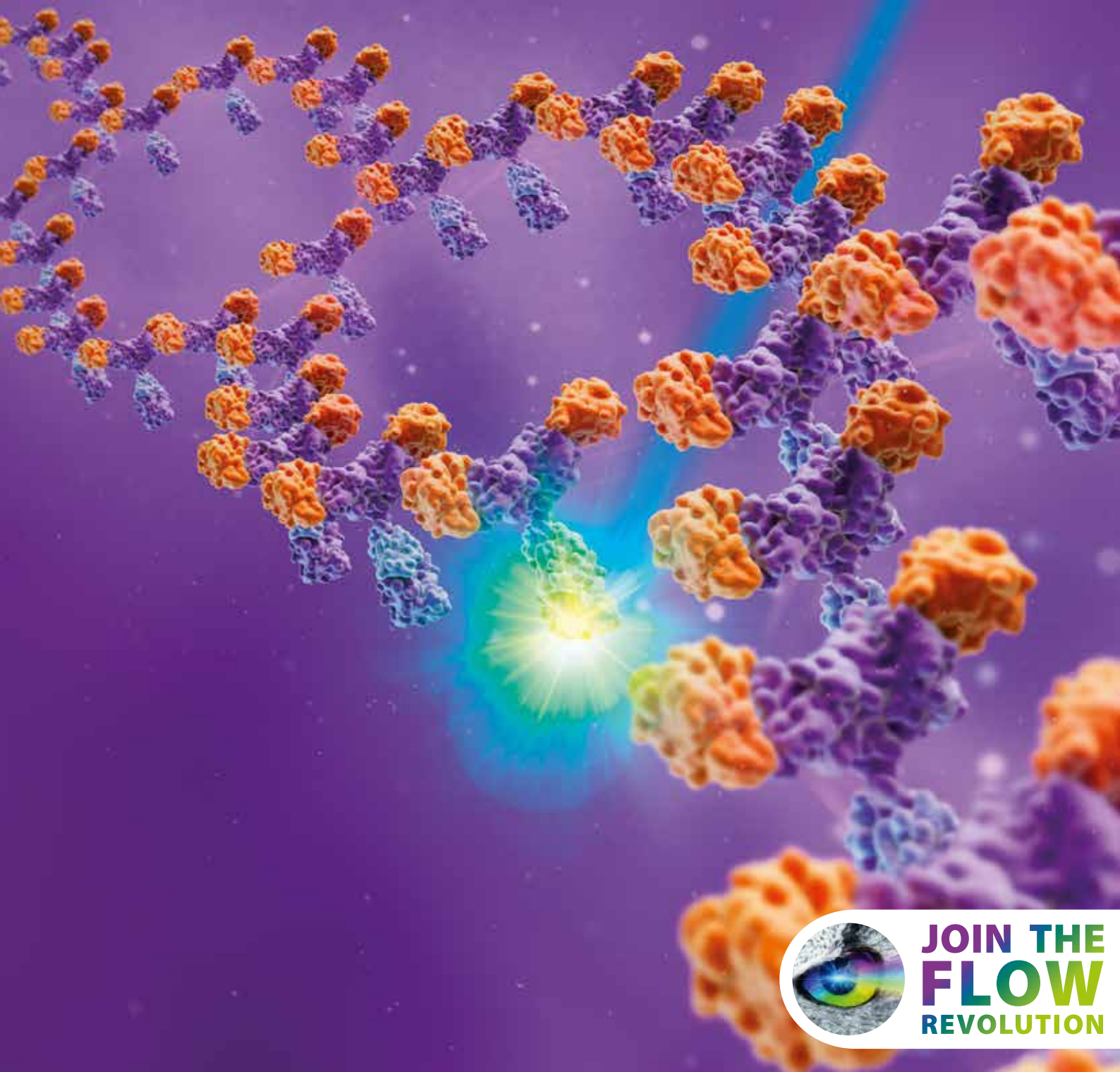
Miltenyi Biotec

REAfinity™ Recombinant Antibodies

Flow cytometry is in their genes



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What are REAfinity™ Antibodies?

REAfinity Antibodies are recombinantly generated antibodies that provide superior lot-to-lot consistency and purity, as compared to mouse or rat monoclonal antibodies. They have been engineered to lack any background binding. Additionally, they all have the same human IgG1 isotype, eliminating any need to include multiple isotype controls during flow analysis

The new way – REAfinity Recombinant Antibodies

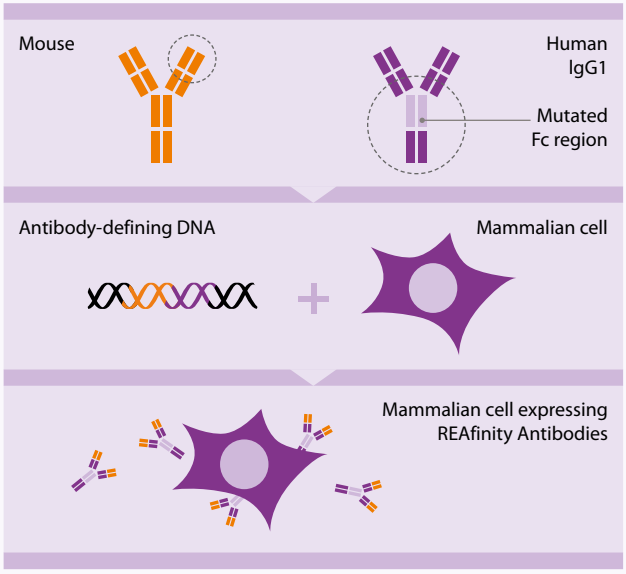


Figure 1: Manufacturing workflow for REAfinity Antibodies

REAfinity Antibodies are generated by cloning the antigen-binding regions from traditional mouse or rat monoclonal antibodies derived from hybridomas with the human IgG1 Fc region. The two genetic regions are fused at the DNA level to create one antibody-defining genetic element (fig. 1). To eliminate background signal during analysis, the human IgG1 Fc region used for generating this fusion gene is mutated, abolishing any binding of the recombinantly engineered antibodies to FcγRs.

To guarantee maximum purity and lot-to-lot consistency, a biologically and chemically defined *in vitro* expression system is used to produce REAfinity Antibodies. The engineered genetic sequence is expressed in a mammalian cell line that is cultured under standardized conditions. To ensure lot-to-lot consistency, the identical cell line is used to produce all REAfinity Antibodies, which eliminates variability due to differences in the expression system.

- High lot-to-lot consistency
- One universal isotype
- No more background signal

REAfinity
Recombinant Antibodies

Made to meet new reproducibility standards

Get reliable results through consistent antibody quality

The manufacturing process of REAfinity™ Antibodies ensures high performance consistency based on the following aspects:

1. REAfinity Antibodies are derived from a defined DNA sequence, which encodes only one type of heavy and light chain, ensuring high antibody purity (fig. 2).
2. Production under biologically and chemically defined, standardized culture conditions in mammalian cells, resulting in high lot-to-lot consistency (fig. 3).
3. Analytical, biochemical, and cell-based testing of every manufactured lot (fig. 3).

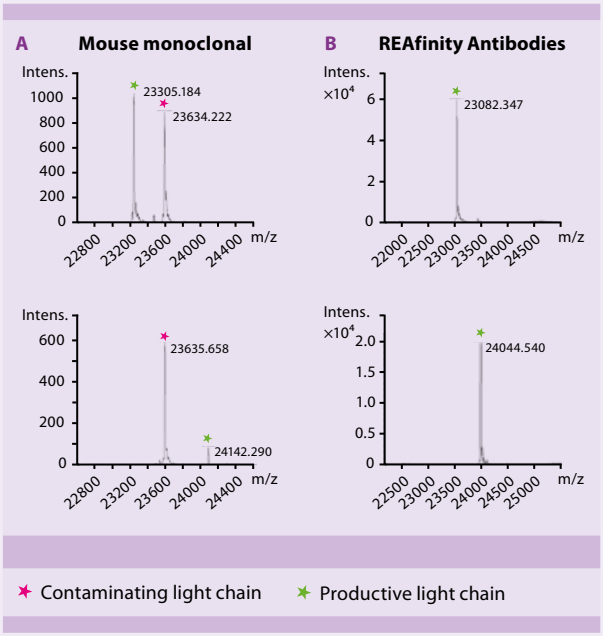


Figure 2: Mass spectrometry analysis of purified antibodies shows that REAfinity Antibodies are defined products, while hybridoma-generated antibodies can be a mixture. (A) Mass spectrometry analysis of two examples of hybridoma-generated monoclonal antibodies shows that both contain a second light chain with a molecular weight of approx. 23,635 Da. In one example bottom left, the amount of the contaminating light chain by far exceeded the productive light chain of approx. 24,142 Da. (B) Two examples of recombinant REAfinity Antibodies show pure light chain populations.

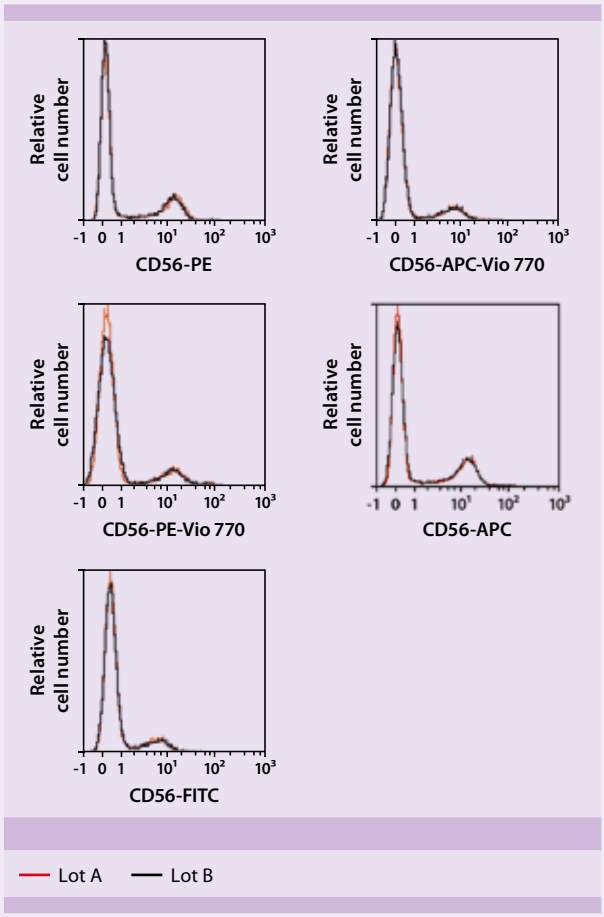


Figure 3: Staining performance of REAfinity Antibodies is nearly identical between different lots. Two manufacturing lots of five different CD56 REAfinity Antibody conjugates were compared by flow cytometry using the MACSQuant® Analyzer. The two histogram curves (black and red) represent the two lots. Lot-to-lot staining performance was nearly identical, when using human peripheral blood mononuclear cells (human PBMCs) from a single donor.

Hassle-free analysis – no more background signal

Analyze what matters

All REAfinity™ Antibodies contain a specifically mutated human IgG1 Fc region that abolishes their binding to Fcγ receptors (fig. 4). This allows for background-free analysis and eliminates the need for additional blocking steps such as using an FcR blocking reagent (fig. 5 and 6).

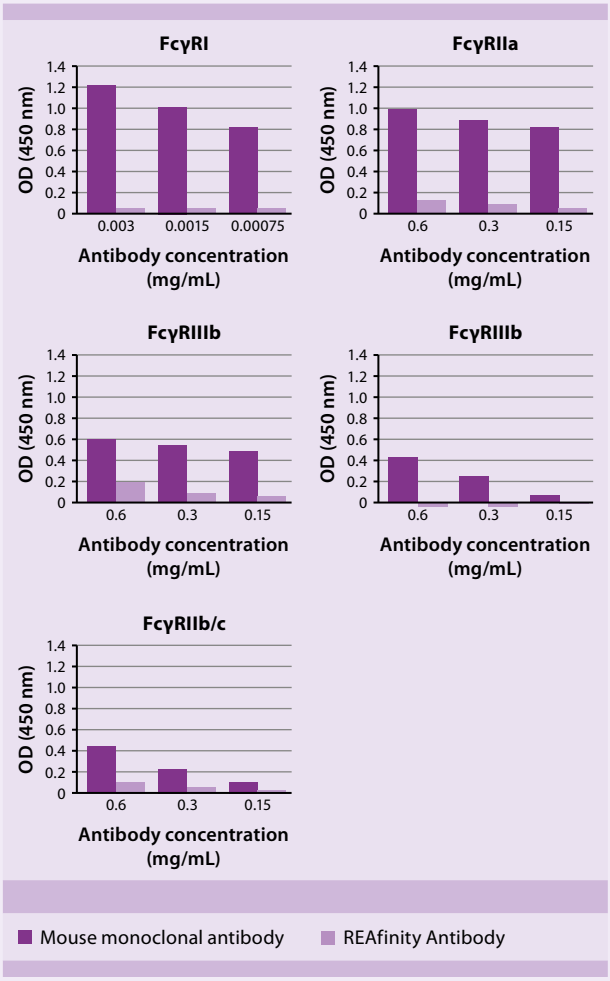


Figure 4: Recombinantly engineered REAfinity Antibodies do not bind to Fcγ receptors. CD144-specific hybridoma-derived mouse monoclonal antibodies (dark purple bars) bind to high affinity FcγRI (CD64) as well as low affinity FcγRIII (CD16) and FcγRII (CD32) receptors. In contrast, REAfinity Antibodies (light purple bars) show virtually no interaction with Fcγ receptors. Binding to five cellular Fcγ receptors was compared using enzyme-linked immunosorbent assays.

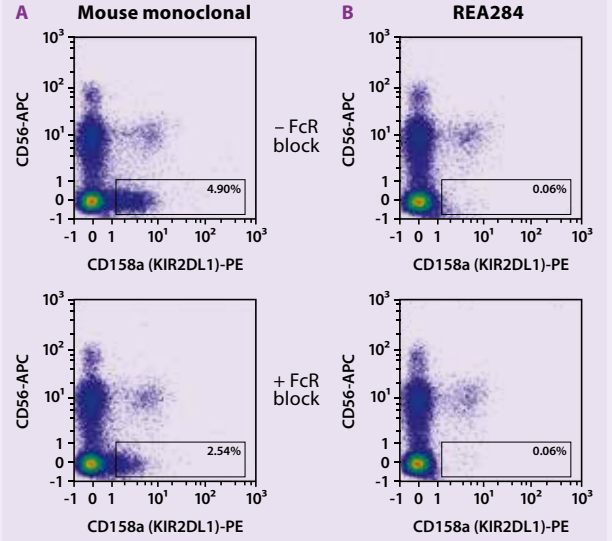


Figure 5: Staining with REAfinity Antibodies shows no background signal, even without FcR block. Human PBMCs were stained with either a mouse monoclonal CD158a-PE antibody or REAfinity CD158a-PE (clone:REA284). Staining was performed without (top) and with (bottom) FcR block. The mouse monoclonal antibody binds unspecifically to CD56-negative cells (A). Staining with the REAfinity Antibody shows no background signal.*

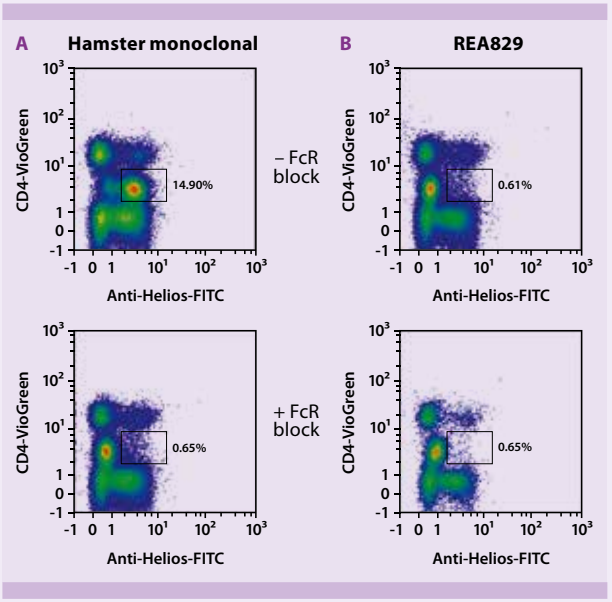


Figure 6: Staining with REAfinity Antibodies shows no background signal, even without FcR block. Human PBMCs were stained with either a hamster monoclonal anti-Helios-FITC antibody or REAfinity anti-Helios-FITC (clone:REA829). Staining was performed without (top) and with (bottom) FcR block. The hamster monoclonal antibody binds unspecifically to FcγR when analyzing the CD4 intermediate monocyte population (A). Staining with the REAfinity Antibody shows no background signal.*

Optimized for flow cytometry applications

The perfect reagents for your cell analysis

REAfinity™ Antibodies are designed with flow cytometry in mind. The following properties ensure their superior performance as compared to traditional hybridoma-derived monoclonal antibodies:

1. Greater purity
2. No background signal
3. Highly optimized and standardized fluorochrome conjugation process

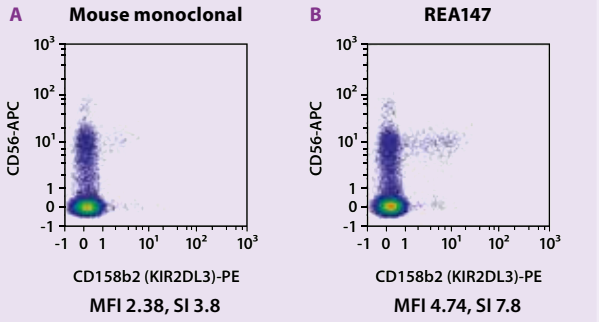


Figure 7: Specific detection of human CD158b2⁺ cells with clone REA147. Human PBMCs were stained with either a PE-conjugated mouse monoclonal antibody (A) or PE-conjugated REAfinity Antibody (B) recognizing CD158b2. Cells were also stained with CD56-APC, human (clone: REA196).*

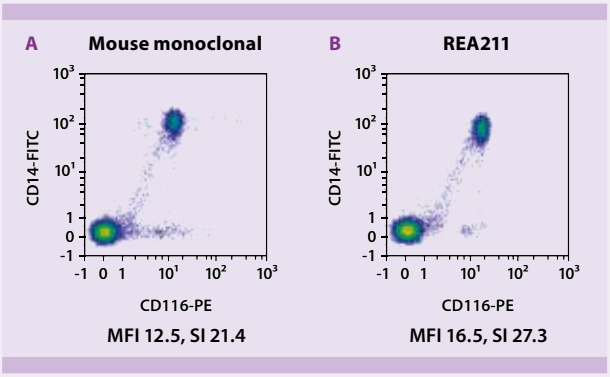


Figure 8: Specific detection of human CD116⁺ cells with clone REA 211. Human PBMCs were stained with either a PE-conjugated mouse monoclonal antibody (A) or PE-conjugated REAfinity Antibody (B) recognizing CD116. Cells were also stained with CD14-FITC.*

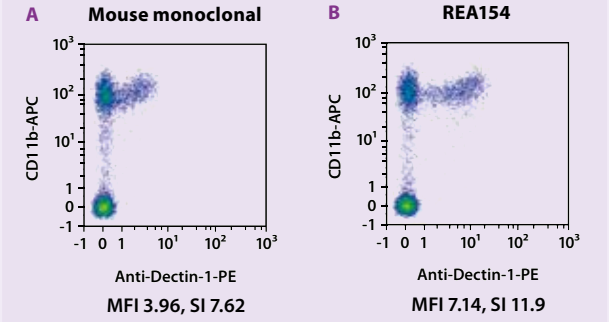


Figure 9: Specific detection of mouse Dectin-1⁺ cells with clone REA154. Bone marrow cells from BALB/c mice were stained with either a PE-conjugated mouse monoclonal (A) or a PE-conjugated REAfinity Antibody recognizing Dectin-1. Cells were also stained with CD11b-APC.*

*Cells were analyzed by flow cytometry on the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

Straightforward experiment planning with only one isotype control

Save efforts when setting up experiments

All REAfinity™ Antibody Clones contain the same specifically mutated human IgG1 sequence as their constant region. This offers the possibility to work with only one type of isotype control, reducing the complexity of experiment planning and saving time.
Hybridoma-derived monoclonal antibodies on the other hand, are composed of antibody isotypes derived from different species and therefore require multiple isotype controls. This complicates not only the management of reagent inventory, but also panel design when setting up experiments.

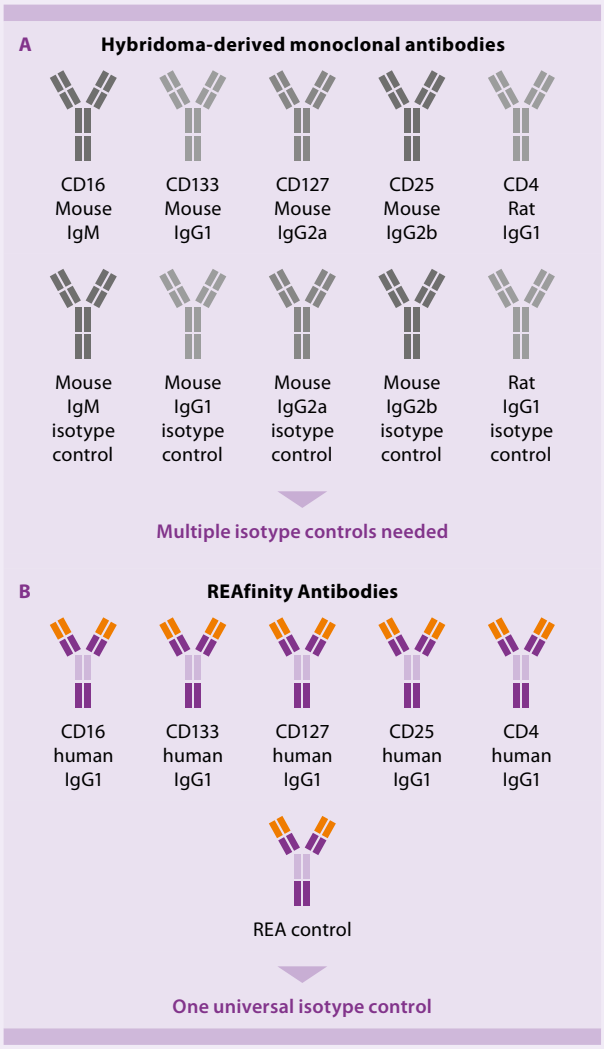


Figure 10: REAfinity Antibodies reduce the complexity of panel design with only one isotype control required.

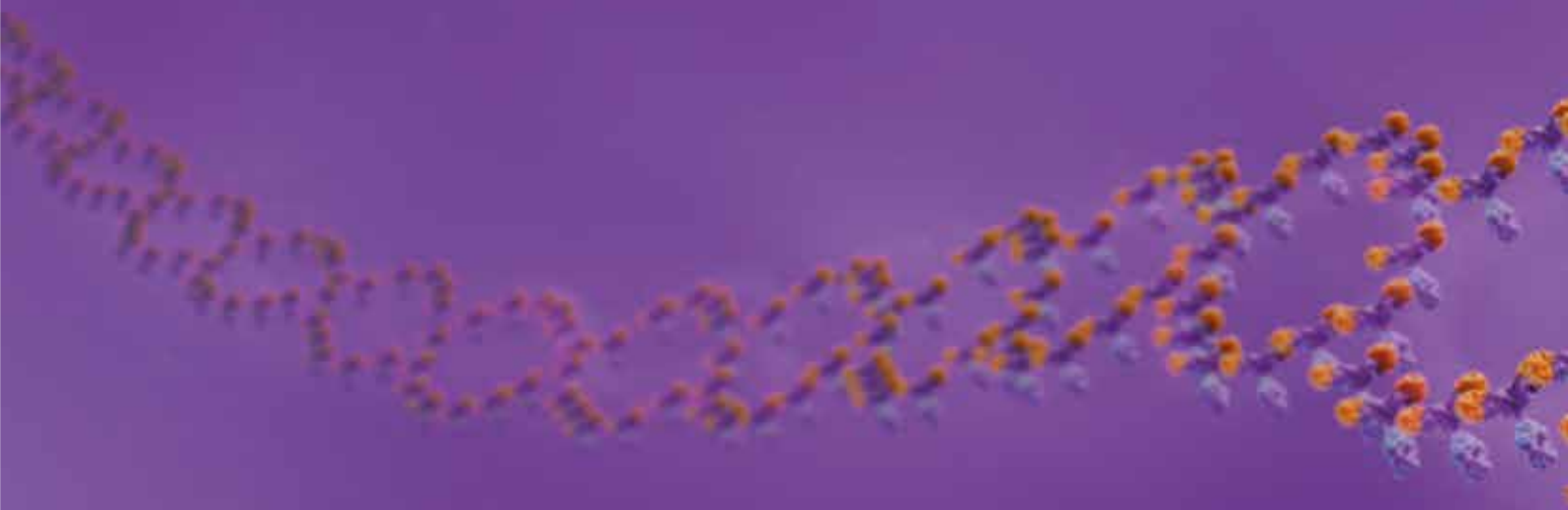
Related products

Isotype control antibodies
All REA clones have human IgG1 as their isotype. Therefore, only one type of isotype control, the REA Control antibodies (clone REA293), is needed. These control antibodies are available for surface antigens (REA Control (S) antibodies) and intracellularly expressed antigens (REA Control (I) antibodies).

REA Control antibodies have the same human IgG1 Fc region as the primary REAfinity Antibodies. Thus REA Control antibodies are highly matched, in their structure and conjugation properties, to primary REAfinity Antibodies. In addition, working with one isotype control reagent saves time and money.

Compensation beads
The MACS® Comp Bead Kit, anti-REA (# 130-104-693) has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated REA clones. After staining with fluorochrome-conjugated REA clones, the MACS Comp Beads, anti-REA are used for automated or manual compensation along with the MACS Comp Beads – blank to check all the negative populations.

▶ miltenyibiotec.com/reafinity



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