

REAFinity™ Antibodies

Frequently asked questions

1. How do I know if the antibody I ordered is a REA clone?

All REA clone names include the term “REA” (figure 1).

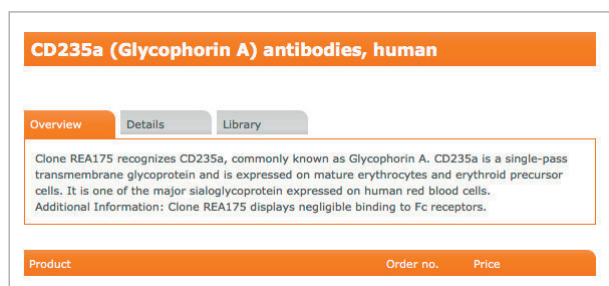


Figure 1: An example of a product page for clone REA175, which recognizes CD235a. In the Overview tab, the clone name contains the term “REA” which means the antibody is part of the REAFinity™ Antibody portfolio.

Additional ways to find REAFinity Antibodies on the Miltenyi Biotec website include:

- Enter the term “REA” in the search field on www.miltenyibiotec.com
- Visit the REAFinity Antibody website at www.miltenyibiotec.com/rea
- Choose “REAFinity Antibodies” in the “Type of antibody” drop-down bar of the Antibody Product Finder (figure 2)

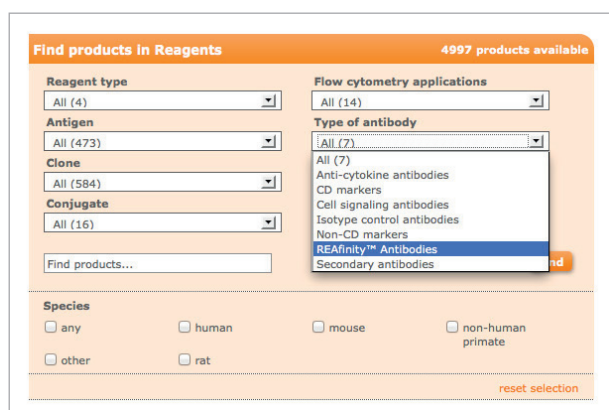


Figure 2: View all “REAFinity Antibodies” in the “Type of antibody” drop-down bar of the Antibody Product Finder.

2. What is a REAFinity Antibody? How does it differ from a standard mouse/rat monoclonal antibody?

REAFinity Antibodies are a new generation of engineered flow antibodies. For each specificity, premium antibody screening technology has been applied to identify the clone with the highest binding affinity and specificity. Only the top candidate is selected to become a recombinant engineered antibody (REA). Based on this process, you can feel confident that your REA clone is the superior antibody choice for your flow experiments.

Additionally, REAFinity Antibodies show virtually no binding to Fcγ receptors (figure 3) which is due to a specific mutation of the Fc part of REAFinity Antibodies. Therefore, no FcR blocking step is required using REA clones and you enjoy significant savings on FcR blocking reagents.

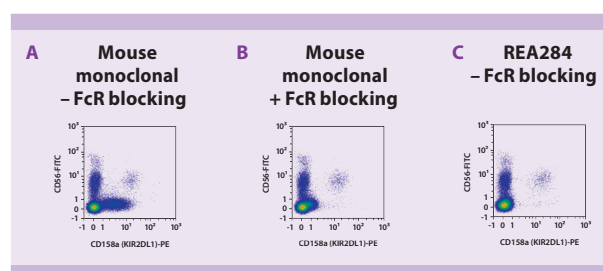


Figure 3: Staining of cells with a mouse monoclonal antibody or a REAFinity Antibody in the absence (-) or presence (+) of FcR blocking reagent. Human PBMCs were stained with either a PE-conjugated mouse monoclonal antibody (A, B) or a PE-conjugated REAFinity Antibody (C) recognizing CD158a. Cells were also stained with CD56-FITC (# 130-100-746) and analyzed by flow cytometry on the MACSQuant® Analyzer. Staining with the mouse monoclonal antibody was performed either with (A) or without (B) pre-treatment with FcR blocking reagent. No FcR blocking reagent was included prior to staining with the REAFinity Antibody (C). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence. Visit www.miltenyibiotec.com/reas to see more performance data.

3. What are the benefits of using REAfinity Antibodies instead of mouse/rat monoclonals?

- No FcR blocking step is required
- Only one REA universal isotype control is needed
- Exceptional purity and lot-to-lot consistency for greater reproducibility
- Less background
- Higher stain index
- Best clones on market

4. In what system are REAfinity Antibodies manufactured?

All REAfinity Antibodies are manufactured in mammalian cells under highly standardized conditions.

5. Why can I use a REAfinity Antibodies without performing Fcy receptor blocking?

All REAfinity Antibodies are human IgG subtype IgG1. In general, IgG1 antibodies show high binding to Fcy receptors. However, in the case of REAfinity Antibodies, the Fc part has been specifically mutated to abolish the undesirable Fcy receptor binding. See the example shown in Question 2: "What is a REAfinity Antibodies?"

6. What isotype control should I use with a REAfinity Antibodies?

The isotype of all REAfinity Antibodies is human IgG1. Therefore only one type of isotype control, named REA Control antibodies (clone REA293), is needed. This control is available both for surface- (REA Control (S) antibodies) and intracellular-expressed (REA Control (I) antibodies) antigens.

You can find the matching control for your experiments at www.miltenyibiotec.com/antibodies

The use of REA Control antibodies (clone REA293) is recommended for surface antigens, even though REAfinity Antibodies show virtually no binding to Fcy receptors. Clone REA293 is used in this case to control for other non-Fcy receptor-mediated unspecific binding of REAfinity Antibodies to cells. Unspecific interactions of the fluorochrome with the cell surface can also be controlled for using conjugated versions of clone REA293.

Note: Isotype controls are mainly used for surface stainings. For intracellular stainings, the isotype control staining should be combined with other negative controls (e.g. staining of unstimulated cells).

As with every isotype control, the amount and concentration of the isotype control used should be the same as for the primary antibody.

7. I was recently told that I do not need isotype controls with REAfinity Antibodies. What has changed?

One main purpose of isotype controls is to show there is no unspecific Fcy receptor-mediated binding. REAfinity Antibodies are specifically engineered to eliminate Fcy receptor binding, so this aspect does not need to be controlled for with an isotype control. However, to exclude other non-Fcy receptor-mediated unspecific binding or unspecific interactions of the fluorochrome we recommend to perform such a control.

8. How do I know if I need REA Control (S) antibodies or REA Control (I) antibodies?

You can find this information on the respective antibody page of your REA clone under the "Related Items" tab.

9. What compensation beads do I use for REA clones?

The MACS® Comp Bead Kit, anti-REA (# 130-104-693) has been developed for optimal compensation of fluorescence spillover of fluorochrome-conjugated REAfinity Antibodies. After staining with fluorochrome-conjugated REAfinity Antibodies the MACS Comp Beads, anti-REA are used for automated or manual compensation along with the MACS Comp Beads – blank for the control of the negative population.

10. How many REA clones are currently available? Will more be added?

There are approximately 200 different REA clones currently available. The number is constantly increasing, so visit www.miltenyibiotec.com/rea for the latest list of available REA clones.

11. I want to use a secondary antibody to amplify the signal of a REAfinity Antibody. Which one should I use?

An anti-Ig κ light chain human secondary antibody should be used because REAfinity Antibodies are human IgG1-κ. You can find the appropriate secondary antibody at www.miltenyibiotec.com/antibodies

12. Are REA clones exclusive for human antigens and target proteins?

REA clones recognize human specificities, mouse specificities, rat specificities, and human/mouse specificities.

13. REAfinity Antibodies are available conjugated to which fluorochromes?

REAfinity Antibodies are available conjugated to the Miltenyi Biotec proprietary **Vio® Dye family** as well as the most commonly used fluorochromes, such as FITC and PE.

The complete fluorochrome offering for REAfinity Antibodies includes:

- FITC
- PE
- APC
- VioBlue®
- VioGreen™
- PE-Vio770™
- APC-Vio770™
- PerCP-Vio700™

Additionally, REAfinity Antibodies are available unconjugated (pure) and conjugated to biotin.

14. How can I increase stain indices for tandem-dye-conjugated REAfinity Antibodies?

Tandem dyes themselves, such as PE-Vio770, PE-Cy™7, PerCP-Vio700, PerCP-Cy5.5, APC-Vio770, and APC-Cy7 tend to bind non-specifically to certain cells, especially to monocytes. Our ready-to-use reagent Tandem Signal Enhancer (# 130-099-888) strongly reduces this non-specific binding. Therefore background signals get decreased, which results in higher stain indices for target cell populations.

15. Do REA clones cost the same as other antibodies from Miltenyi Biotec?

Yes. REAfinity Antibodies have the same competitive pricing as the standard mouse/rat monoclonal antibodies.

16. Are REAfinity Antibodies part of the Miltenyi Biotec custom antibody and bulk offerings?

Yes. REA clones are part of the extensive Miltenyi Biotec **Custom Antibody Design Service**.

You can select any antibody from the MACS Antibody portfolio and the fluorochrome to which it should be conjugated. Additionally, you can also choose the desired concentration (up to 5 mg/mL), buffer composition, and antibody format (e.g. in solution, lyophilized, or as part of a cocktail).

17. Can I request a custom REA clone for my specificity of choice?

Currently the option does not exist to generate exclusive REA clones for customers. However, we are interested in hearing your suggestions to help us support your research needs.

Do you need more support? Contact the Technical Support Team at macstec@miltenyibiotec.de or go to www.miltenyibiotec.com/support



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