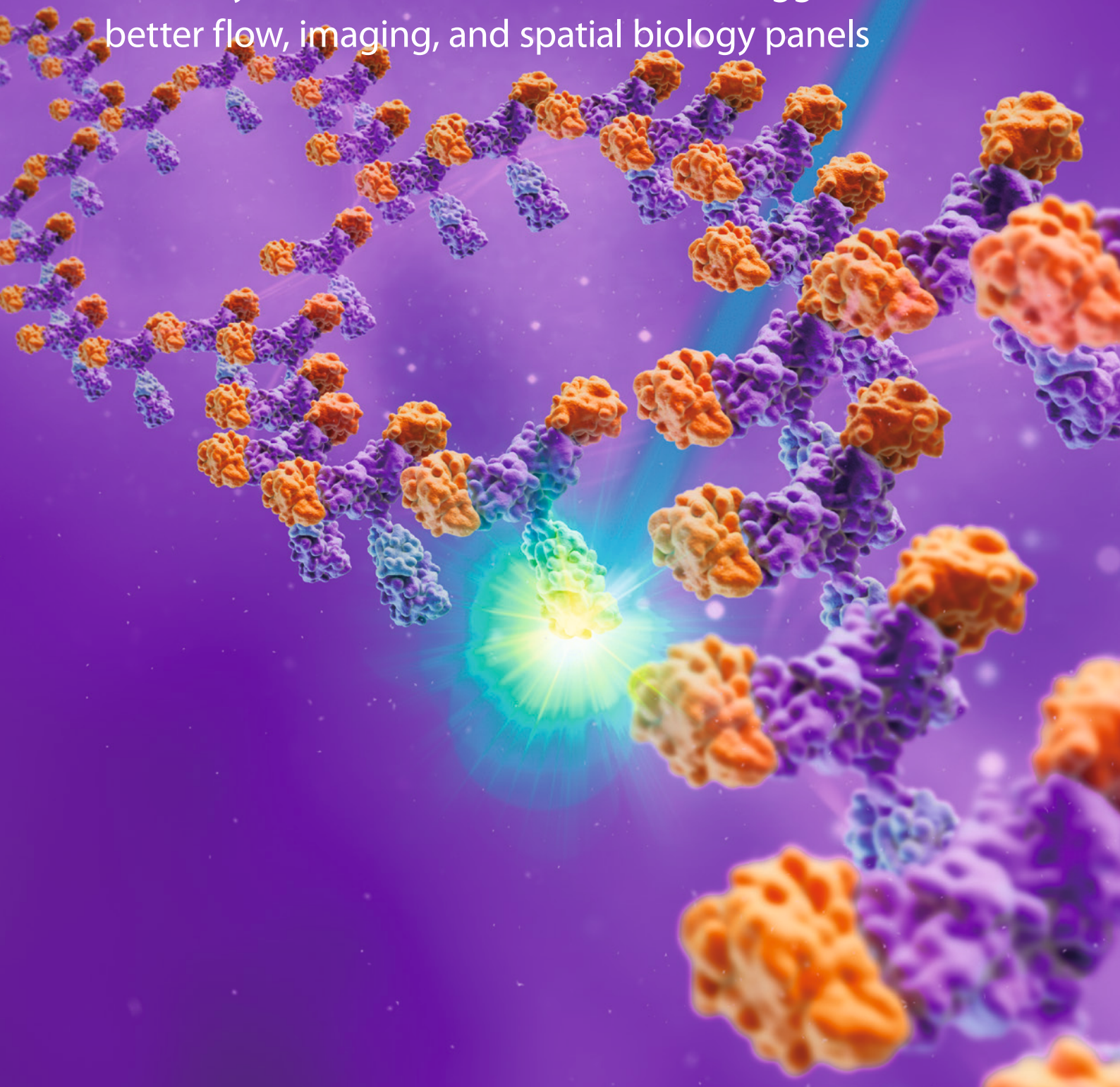
Miltenyi Biotec

The last word in reliability

REAfinity® Recombinant Antibodies for bigger, better flow, imaging, and spatial biology panels





- Extensively tested and optimized
- Huge range of pretested, customizable antibodies
- ISO 9001–certified quality management system

REAfinity
Recombinant Antibodies

Say goodbye to outdated hybridomas

Antibodies that do the job right first time, and every time

More than 17,000 REAfinity Recombinant Antibody conjugates let you focus on the scientific aspects that truly matter.

At Miltenyi Biotec, we opt for recombinant antibodies, which enable sequence verification and optimization for lot-to-lot consistency, making them superior to hybridoma-based antibodies.

Our investment in innovation to advance science

As far back as 2015, more than 100 of the world's leading scientists collectively called for better standards and reproducibility in antibody production, proposing recombinant antibodies to circumvent variability.[1]

Recombinant antibodies: precision-engineered for consistency, reliability, and ethics

Recombinant antibodies are created by cloning antibody genes into expression systems, typically using bacteria, yeast, or mammalian cells. At Miltenyi Biotec, we use mammalian cells for proper folding, glycosylation, and functionality, closely resembling natural antibodies. We also proactively perform additional analyses on existing hybridoma-based antibodies, giving rise to sequences that contribute to further novel recombinants. Unlike hybridoma methods, recombinant antibodies maintain high specificity, low immunogenicity, and consistent functionality over time, without the need for animal immunization, supporting the 3R Principle (Replace, Reduce, Refine).

With the use of antibodies in basic research, translational, and clinical settings being as ubiquitous as the air we breathe, it makes sense to avail yourself of the very highest quality.

Choosing REAfinity Recombinant Antibodies for your application means you get to benefit from:

- Unmatched specificity and sensitivity – precisely tag only your target cells with minimal background, thanks to Fc region engineering.
- Rock-solid reproducibility – consistent results year after year, powered by a fully defined *in vitro* expression system.
- Background-minimizing dyes – uniquely engineered Vio® Dyes for clean, clear staining.
- No more isotype controls – standardized to a single human IgG1 backbone.
- Expert-designed panels and kits – tested and ready for high-impact applications.
- Antibodies that are optimized for your workflow – validated for flow cytometry and 3D immunofluorescence applications, and pretested for spatial biology runs.
- Out-of-the-box readiness – sample compatibility pretested so you can jump straight to panel building.
- Endless customization – tailor solutions to your exact needs.
- 24/5 scientific support – real experts, real help, when you need it.

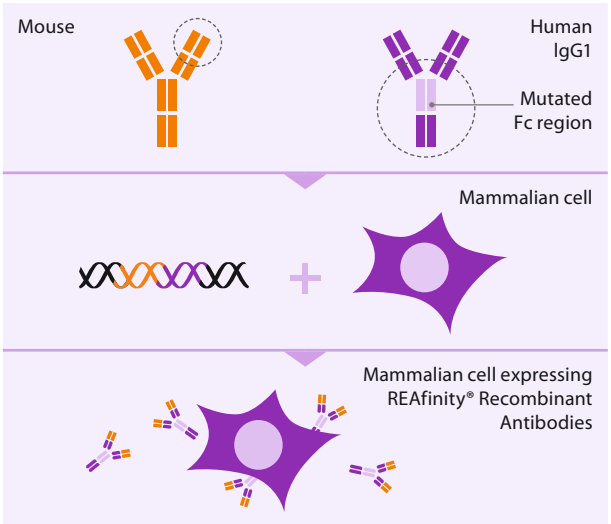


Figure 1: REAfinity Recombinant Antibodies are produced in the manner described above.

Extended validation for reliable and accurate results

Specificity validation you can believe in

During development, we perform multiple validation tests to verify the quality and consistency of REAfinity Antibodies. We also provide extended validation data, highlighting details of performance, specificity, and fixation compatibility. These antibodies are indicated with the extended validation stamp. Below follow some examples of how we guarantee the superior quality of our antibodies.

Knockout or knockdown of the target protein

This method leaves no room for ambiguity: the target gene is decisively knocked out in a validated cell line using precise, site-specific nucleases, with knockout confirmation by direct sequencing of the target locus. An antibody that fails to bind to these knockout cells provides definitive proof of epitope specificity. We further reinforce this validation with high-resolution fluorescence microscopy and quantitative flow cytometry, ensuring absolute confidence in antibody performance.

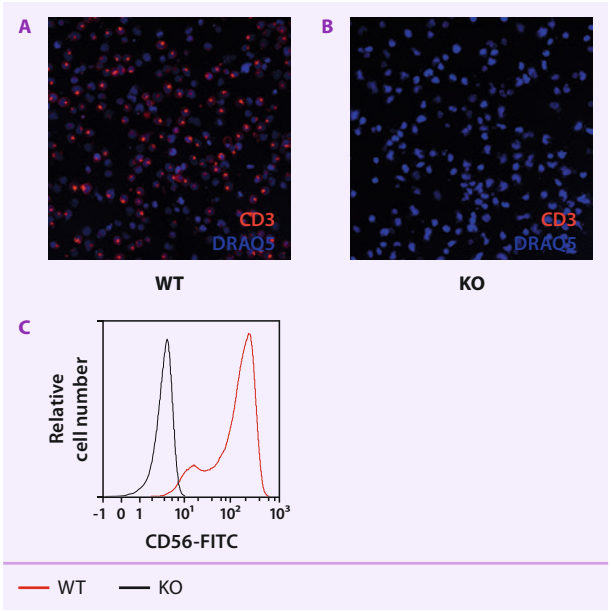


Figure 2: Fluorescence microscopy image of CD3 knockout cells. Wild type (left) and knockout cells (right) were stained with CD3-PE (REA613, red) and counterstained with DRAQ5 (blue) as DNA stain. Flow cytometry was performed with the MACSQuant® Analyzer. Cell debris, dead cells, and cell doublets were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Similar to knockout, the knockdown of the target protein serves as an approach to validate antibody specificity. Using RNA interference, the translation of the target RNA is inhibited by transfecting cells with small non-coding RNA oligonucleotides. A comparison between the transfected cells with the control cells reveals the specificity of the tested antibody to its antigen.

Counterstaining

To confirm antibody specificity in tissue sections, stainings with suitable counterstainings are performed. The counterstain provides context in the tissue so that it is easier to identify target cell population and verify the primary stain. The test is suitable for fluorescent IHC.

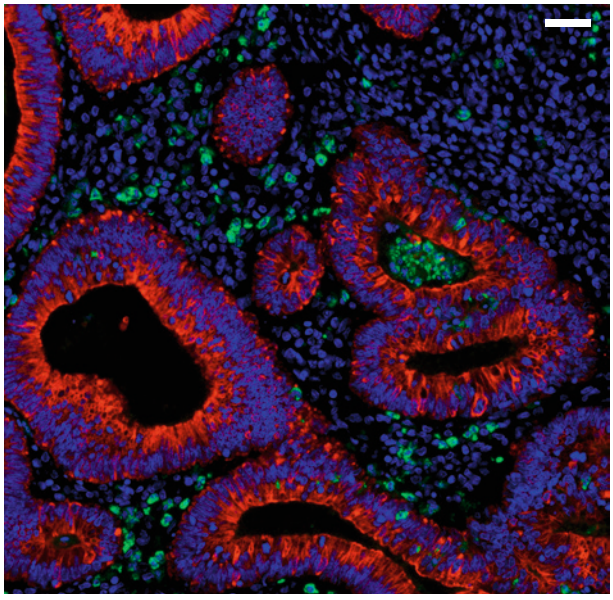


Figure 3: Human colorectal carcinoma section (FFPE) stained for neutrophile marker CD66b (green) with Cytokeratin-LMW counterstain (red). Scale bar is 30 µm.

Performance comparison
We rigorously benchmark the performance of our conjugated antibodies against other leading suppliers to guarantee equal or superior efficacy. Every production lot undergoes stringent validation through a comprehensive dilution series, ensuring consistent, precise performance within the defined antibody titer range – no exceptions.

Compatibility with fixation
Our antibodies are also tested for their stability during fixation to verify that the epitope of an antigen can still be detected after the process. Compatibility information can be found on the product page and within the application section and in the extended validation tab.

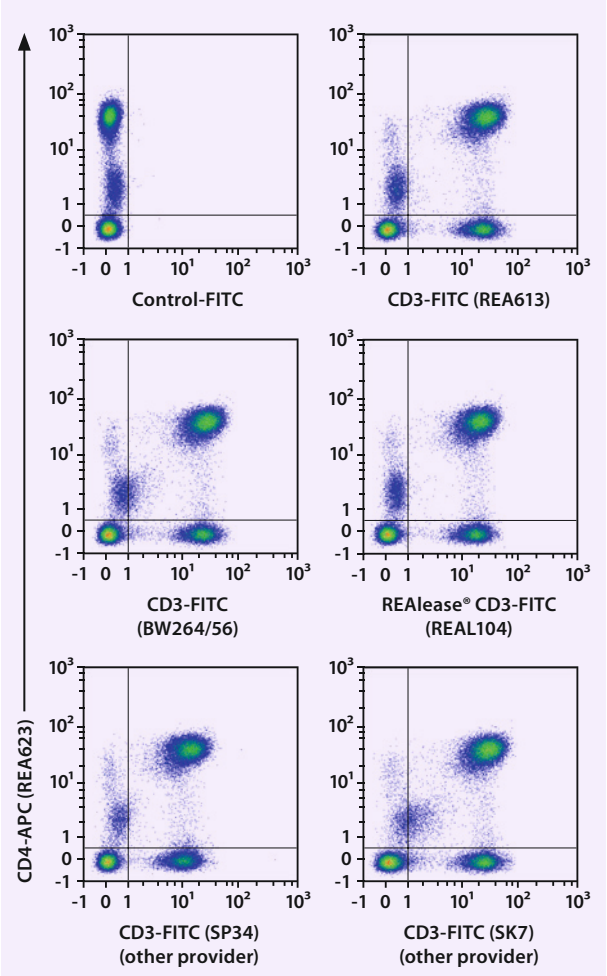


Figure 4: Flow cytometric comparison of different clones for CD3. Human peripheral blood mononuclear cells (PBMCs) were stained with CD3 antibodies and with a suitable counterstaining. As a control, CD3 antibody staining was omitted and cells were measured in the same channels. Flow cytometry was performed with the MACSQuant Analyzer. Cell debris, dead cells, and cell doublets were excluded from the analysis based on scatter signals and 4',6-diamidino-2-phenylindole (DAPI) fluorescence. No FcR blocking reagent was used. The recommended titers of respective antibodies from different suppliers were used.

Vio Dyes: the perfect partner to REAfinity Antibodies

Vio Dye Fluorophores

With Vio and Vio Bright Dyes, you're not just using another set of fluorescent dyes, you're unlocking the full potential of your research. Designed for both flow cytometry and imaging, Vio and Vio Bright Dyes support the inherent need for innovative fluorochromes to meet the growing demand for sophisticated multi-color analysis, offering a wider spectral range and exceptional signal discrimination. Whether you're imaging tissues or analyzing cell populations, Vio and Vio Bright Dyes provide the versatility and performance to meet your most demanding research needs, so you can go beyond the limits of traditional fluorochromes.

1. Design large multi-color antibody panels: utilize the full potential of your instruments.
2. Streamline antibody panel design: simplify your multi-color panel design through better separation and reduced spillover.
3. Enjoy more flexibility: design antibody panels that give flexibility for future experiments.

Advantages of Vio Dyes

The Vio and Vio Bright Dyes were created to improve all aspects involved with flow cytometry and fluorescence microscopy. These include:

- Low spillover due to spectral separation/large stokes shift: no need for extensive compensation.
- High signal-to-noise ratio.
- Low background.

25% decreased spillover

Vio Dyes show reduced spillover into adjacent detection channels of up to 25%, meaning less activation of detectors other than the ones intended for the given wavelength, thus reducing the need for compensation.

Enhanced brightness

Stain indices and mean fluorescent intensities of Vio Dyes are higher than those of comparable dyes, allowing for an improved resolution of positive and negative populations. This is also ideal for the detection of markers with low expression levels.

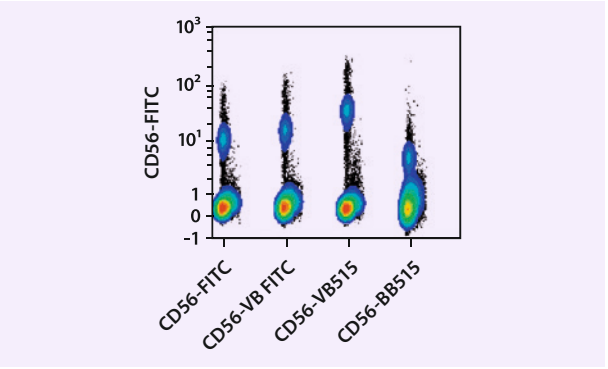


Figure 5: Human PBMCs were stained with CD56 antibodies conjugated to FITC, Vio Bright FITC, and Vio Bright 515. VB: Vio Bright; BB: Brilliant Blue. Enhanced brightness can clearly be seen.

Photostability

Vio Dyes have improved or comparable photostability to alternatives, often a limiting factor in many applications of fluorescence microscopy.

Fixation stability

Vio Dyes show excellent stability toward methanol- and paraformaldehyde-based fixatives, with only little compromise in brightness.

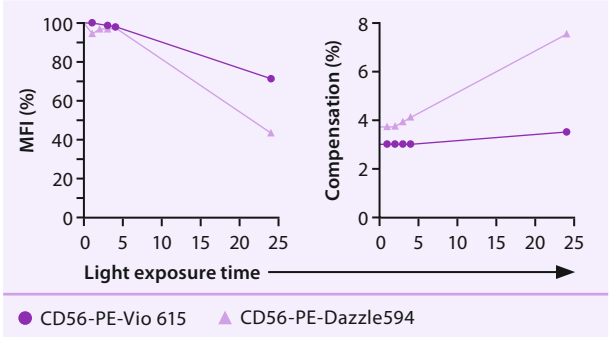


Figure 6: Human PBMCs were stained with CD56 antibodies conjugated to PE-Vio 615 (clone REA196) or PE-Dazzle 594 (clone HCD56). Stained cells were then exposed to ambient light (~850 lux) and analyzed at different time points by flow cytometry using the MACSQuant VYB. Spillover into the Y1 channel, which is optimized for detection of the PE signal, was also analyzed after exposure to light.

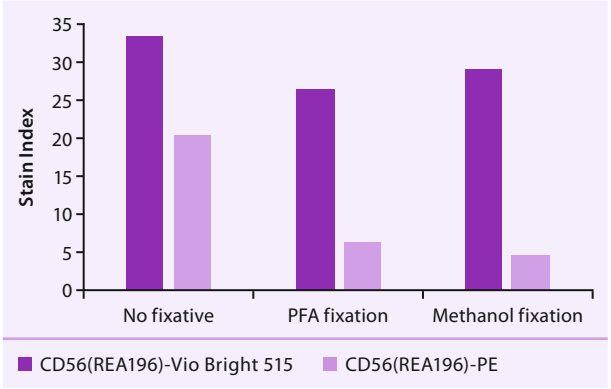


Figure 7: Human PBMCs were stained using a CD56 antibody (clone REA196) conjugated to Vio Bright 515 or PE. Vio Bright 515 showed better signal after fixation with paraformaldehyde (PFA) or 90% methanol compared to PE.

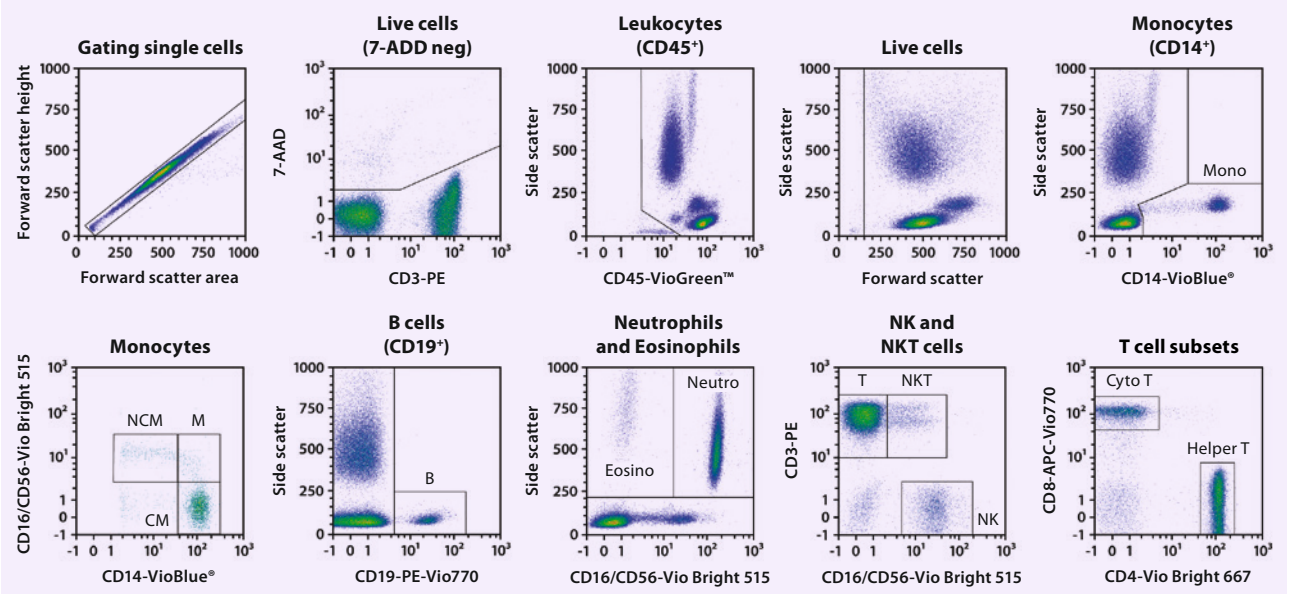


Figure 8: The 8-Color Immunophenotyping Kit, anti-human, REAfinity is an advanced TBNK reagent to determine frequencies of immune cell populations: T cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), NKT cells ($CD3^+CD56^+$), NK cells ($CD3^-CD56^+$), B cells ($CD19^+$), non-classical monocytes ($CD14^+/CD16^{++}$), intermediate monocytes ($CD14^{++}/CD16^+$), classical monocytes ($CD14^+/CD16^-$), neutrophils ($CD16^+/SSC^{high}$), and eosinophils ($CD14dimCD16^-/SSC^{high}$) in whole blood, PBMCs, or other human samples by flow cytometry. The kit includes a ready-to-use phenotyping cocktail, single antibodies for compensation, and Red Blood Cell Lysis Solution.

Antibodies for every occasion: no more switching

Antibodies and kits designed for various applications

At Miltenyi Biotec, we provide high-quality recombinant antibodies designed for precision and reliability across a range of applications. From flow cytometry analysis and sorting to mass cytometry, spatial biology, and imaging, our antibodies deliver superior performance, consistency, and reproducibility. Backed by cutting-edge technology and rigorous quality control, our products empower researchers with the accuracy they need for groundbreaking discoveries.

Antibodies for mass cytometry

Greater flexibility and higher consistency in your mass cytometry experiments seem mutually exclusive? Our antibodies for mass cytometry will convince you otherwise: A large portfolio of recombinant clones for consistent conjugation performance and reproducible results. All provided in a concentration of 100 µg / 100 µL and in a stabilizer-free aqueous buffer, which makes them ready-to-conjugate to your isotope of choice.

Custom Antibody Design Service

Our Custom Antibody Design Service (CADS) enables researchers to tailor personalized solutions for their flow cytometry applications, including pure, unlabeled antibodies, single- and multi-color antibody conjugates, and multi-color cocktails. The CADS team provides high-quality and affordable reagents made to your specifications.

Antibodies and kits designed for various flow cytometry and sorting applications

We have more than 17,000 flow cytometry and flow sorting antibody conjugates designed for high specificity and bright signals. They comprise primary and secondary isotype control antibodies, Viobility™ Dyes, and ready-to-use kits for all key applications, including immuno-oncology, neuroscience, and cytokine detection. Due to their basis in recombinant technology, these reagents allow high lot-to-lot consistency and background-free cell analysis or cell sorting. Thoroughly tested and validated for all flow applications, REAfinity Recombinant Antibodies and REAlease Releasable Antibodies ensure high standardization and reliable results.

LEARN MORE

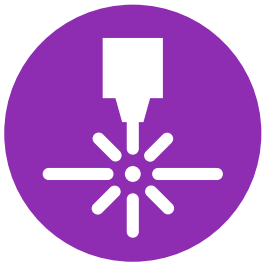
8-Color Immunophenotyping Kit, anti-human, REAfinity

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LEARN MORE

Discover our custom antibody and antibody panel service for your unique needs at

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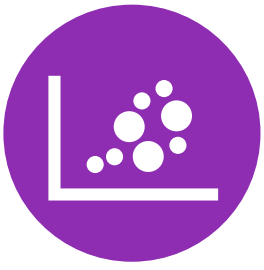
Flow/Sorting



Cyclic staining



IF



Mass cytometry

Flow cytometry: antibody panel building at the push of a button

MACS® Flow Cytometry – Multicolor Panel Builder

Choose the best antibodies for your multicolor flow cytometry experiments and easily create your panels in just a few steps. Design your panel from our large portfolio of REAfinity Antibodies and unique Vio Dyes. See the spectral overlap in one easy overview per laser, and check potential spillover in the Fluorochromes Spillover Matrix.

- One-click panel building with our new, advanced panel-building tool.
- Save time with tailor-made panel recommendations for your flow cytometry assays.
- Combine the benefits of manual panel design and fast, automated assistance.

LEARN MORE

Explore how easy antibody panel design can be at

▸ **miltenyibiotec.com/antibody-panel-builder**

Pretested antibody panels – Miltenyi Biotec Tested Panels (MBTPs)

Designing the perfect combination of markers and dyes for flow cytometry is complex and high-stakes. One wrong move can cost you valuable time, resources, and samples. That's why we developed MBTPs – optimized, standardized antibody panels that ensure reliable, reproducible results, saving researchers both time and effort.

Validated by our in-house experts and trusted by scientists worldwide, MBTPs remove the guesswork from panel design and deliver consistent, high-quality data with confidence.

- All essential reagents included – everything you need, nothing you don't.
- Clear, step-by-step staining protocols – designed for speed and precision.
- Smart, intuitive gating strategies – for clean, confident analysis.

MBTPs simplify your workflow and supercharge your results, so you can focus on discovery, not troubleshooting.

LEARN MORE

Find ready-made panels fitting your research at

▸ **miltenyibiotec.com/multicolor-flowcytometry-panels**

Imaging and spatial biology

Fluorochrome-conjugated antibodies for your imaging and spatial biology needs

You need a clear picture of what's going on, regardless of your weapon of choice: From standard fluorescence microscopy, to light sheet imaging of large volumes, or high-plex spatial biology. Choose from the largest portfolio of antibodies available that suit your sample just right: our fluorochrome-conjugated MACS Antibodies.

Browse our large portfolio of immunofluorescent antibodies for antigen-specific and bright signals, reduced staining time, and minimal background. With an ever-increasing range of markers and sophisticated recombinant antibody technologies, our imaging reagents pave the way to new insights.

3D-IF–validated antibodies

Miltenyi Biotec's 3D immunofluorescence (3D-IF) validated antibodies are optimized for deep tissue penetration of large cleared samples, addressing the challenge of achieving complete antibody penetration in thick tissue sections or entire organs. Our antibodies are validated for functionality with MACS Clearing and MACS Deep Clearing Kits, and are frequently reported to be compatible with various tissue clearing methods.

1. The validated 3D-IF antibodies enable deep tissue labeling across a wide size range, from organoid to entire mouse brain.
2. Being fluorochrome-conjugated, they cut staining time by 50%, eliminating secondary antibody staining and significantly speeding up immunostaining and sample preparation.
3. Furthermore, our antibodies are conjugated to bright and photostable Vio Dyes in four channels, Vio B515, Vio G570, Vio R667, and Vio 780, ensuring maximum imaging flexibility.

Pretested antibodies for spatial biology: MACSima™ Imaging Cyclic Staining (MICS)

The MACSima Platform utilizes MICS, a technology that allows the capturing of microscopy data from hundreds of markers in the same section. With this approach, the sample is first stained with multiple fluorochrome-conjugated antibodies and then imaged. Afterward, the fluorescent signal is erased and the next cycle can start.

We pretest our antibodies for use with MICS. Our extensive portfolio of MICS antibodies, covering 640 markers with more than 2,400 individual antibodies, is tested on FFPE and frozen sections of human and mouse reference tissues for compatibility. This pretesting greatly reduces the time required to set up a spatial biology panel and the amount of necessary preliminary experiments, allowing you to focus on your research question.

Spatial biology antibody panels

Jump-start your experiments with our pretested panels for spatial biology

Spend less time building the foundations and focus on your research with our pretested panels. Our REAplex Immuno-oncology Core Panel for spatial biology covers the essential markers to visualize the tumor microenvironment in a predefined panel that comes with optimized imaging and staining parameters, and provides guidance from experiment setup to data analysis. To this foundation, you can flexibly add any marker relevant to your scientific question.

Custom antibody panels for MICS

REAscreen™ Design provides ready-to-use 96-well plates containing a user-defined panel of dried fluorochrome-conjugated antibodies. In combination with the MACSima Platform, REAscreen Design enables convenient, standardized multiplex imaging.

LEARN MORE

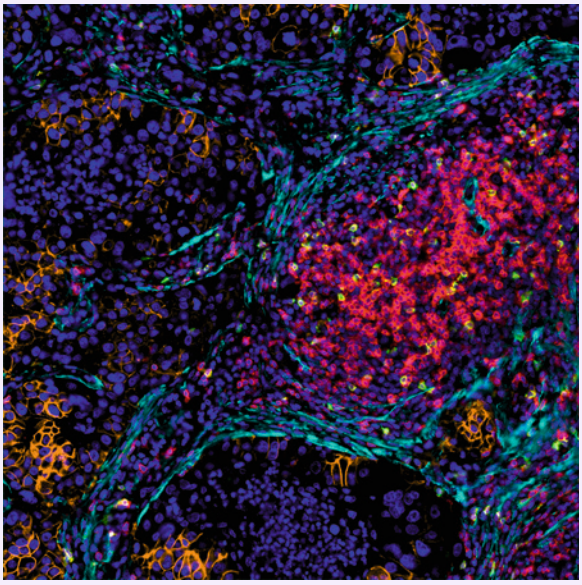
Find customized antibody panels tailored to your MACSima Platform spatial biology experiment at

▸ **miltenyibiotec.com/customized-antibodypanels-MICS**

References

1. Baker, M. (2015) Reproducibility crisis: Blame it on the antibodies. Nature 521: 274–276. https://doi.org/10.1038/521274a

REAplex Immuno-oncology Core Panel



Spatial mapping and profiling of immune and tumor cells is crucial to understanding the complex tissue architecture and the interactions in the tumor microenvironment. The REAplex Immuno-oncology Core Antibody Panel on the MACSima Platform represents a multiplex IHC-like approach, identifying the cells within your tissue sample, unveiling their spatial arrangement and interactions. This pretested panel of 20 curated markers allows you to:

- Visualize immune cells within their tissue context: identify invading immune cells like macrophages, T, B, and NK cells.
- Identify tumor and healthy tissue: stain for CD326 (EpCAM) and cytokeratins to visualize epithelial tissue and assess the degree of invasiveness.
- Explore tumor activity and immune regulation: identify which cells are dying or proliferating. Visualize the tumor's neovascularization. Assess immune regulation through the PD-1/PD-L1 pathway.

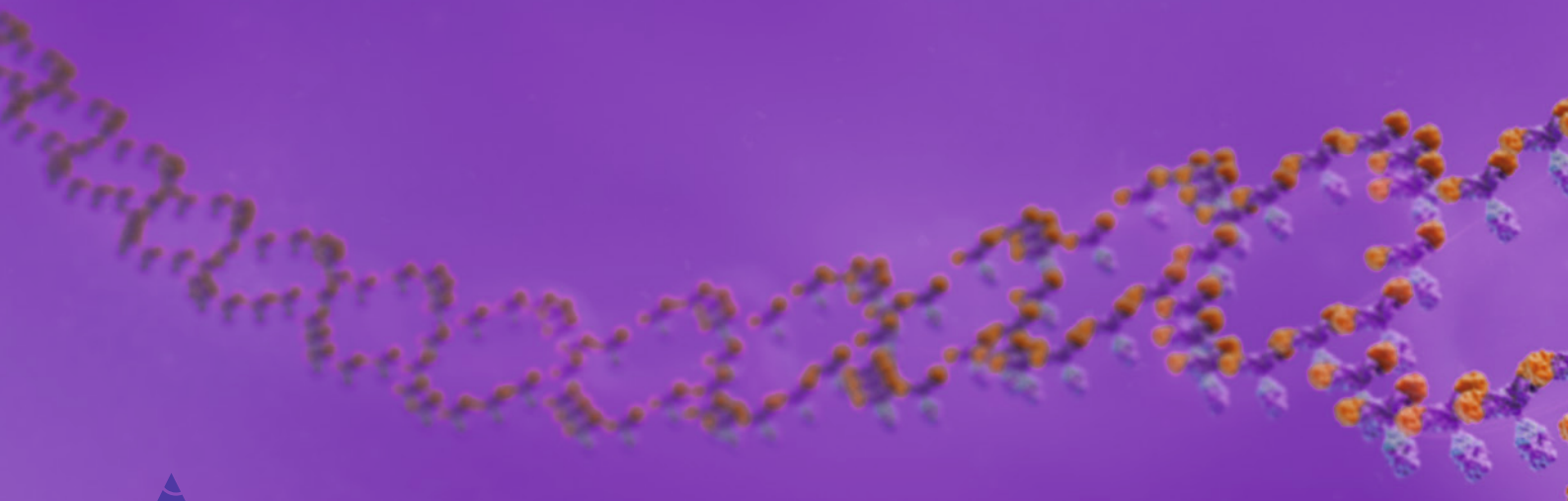
Figure 9: FFPE section of lung tissue from a human donor with non-small-cell lung cancer, stained with the REAplex Immuno-oncology Core Panel. Shown are channels for CD3 (red), CD8a (green), EpCAM (orange), and Actin (turqouise).

LEARN MORE

Predefined REAplex Antibody Panels

▸ **miltenyibiotec.com/predefined-REAplex-antibodypanels**

▸ miltenyibiotec.com/reafinity



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