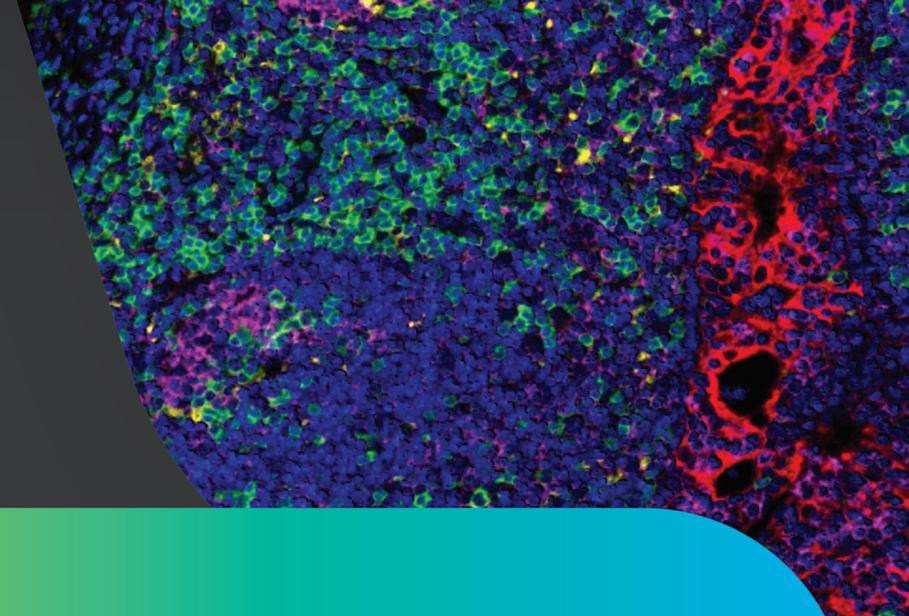




Complexity of the Tumor Microenvironment



Radiant reproducibility

Get results you can rely on with antibodies that are 100% guaranteed to work

The best discoveries take determination, dedication and reliable antibody tools. Yet, nearly 50% of all antibodies fail.* Backed by more than 45 years of experience in manufacturing and validation, you can trust every antibody from Bethyl. From catalog and custom polyclonal and recombinant rabbit monoclonal antibodies, to ELISA kits and sets, we have the solutions you need to take it to the next level. Work with Bethyl to bring your discovery into full focus.

See our data at bethyl.com/breakthrough

Since 1972, Bethyl Laboratories has been dedicated to improving lives by supporting scientific discovery through its qualified polyclonal and recombinant rabbit monoclonal antibody products and ELISA kits. Every antibody Bethyl sells has been manufactured and validated on site in the USA to exacting standards. Our in-house validation process ensures target specificity and sensitivity to provide confidence in your results. With over 40 years of experience, Bethyl is also a leading provider of bulk and custom production services. Bethyl's newest catalog offering of recombinant rabbit monoclonal antibodies serve to advance immuno-oncology research. To learn more, please visit www.bethyl.com. We put a lot in every drop.

Multiplex IHC: Making Discoveries Multicolor

Brilliant visualization

See the difference to make big breakthroughs in your tumor microenvironment research

In the world of next generation immuno-oncology research, having confidence in your immunoassay results is vital. Unfortunately, 75% of antibodies in today's market are non-specific or simply do not work at all.* That's why at Bethyl, we manufacture and validate every antibody on-site to ensure target specificity and sensitivity. More than 10,000 independent citations over the past 15 years have proven that our antibodies will function as designed in your assay — and we offer a 100% guarantee. Work with Bethyl to bring your discovery into full focus.

See our data at bethyl.com/immuno-oncology



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ADVANTAGES OF MULTIPLEX IHC



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Really Good Antibodies

*Weller, MG, Analytical Chemistry Insights:11, 21-27 (2016).
Antibodies shown: CD3E (A700-016) & CD20 (A500-017A)
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Multiplex IHC: Making Discoveries Multicolor

Complexity of the Tumor Microenvironment

The tumor microenvironment (TME) is a complex mass of malignant and nonmalignant cells, signaling molecules, extracellular matrix, and blood vessels. Immunomodulation of the T-cell response within the TME, via inhibition of immune checkpoints and co-inhibitory molecules such as CTLA-4 and PD-1, is a promising cancer therapy. Multiplex immunohistochemistry (mIHC) enables the tracking of multiple markers within the TME, predicting therapeutic response and highlighting new therapeutic targets.

TME Expression Profiles

TME expression profiles guide understanding of the interactions between malignant and nonmalignant cells.

T cells: CD3, CD4, PD-1, CTLA-4, FOXP3, CD4, granzyme B, granzyme A, CD25, CD39, CD73, CD103

Extracellular matrix: collagen, fibronectin, laminin

Macrophages: CD68, HLA-DR, CD14, CD11b, CD163, CX3CR1

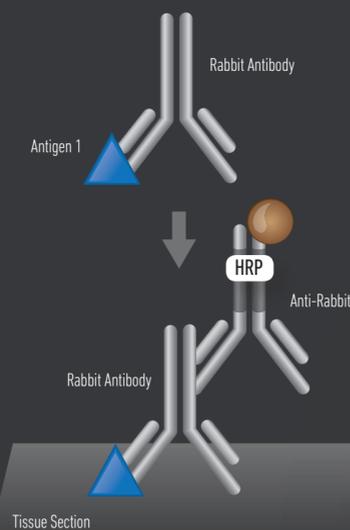
B cells: CD19, CD20, CD40, CD80, CD86, CD69

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EXPLORING LIFE, INSPIRING INNOVATION

Traditional One-Color IHC

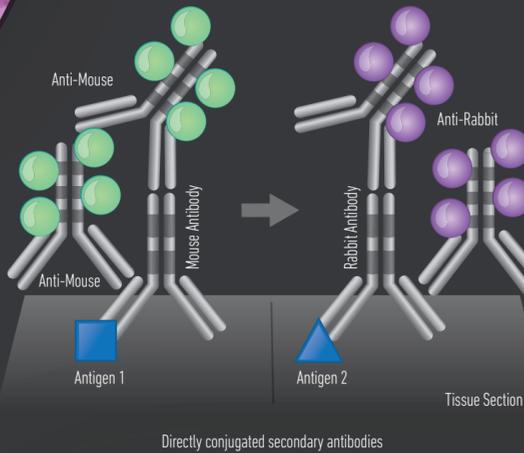
Traditional, one-color IHC cannot differentiate between multiple cell types and targets in tumors. To immunostain more than two targets, serial sections or serial staining procedures must be used.

One-Color Process



Traditional mIHC

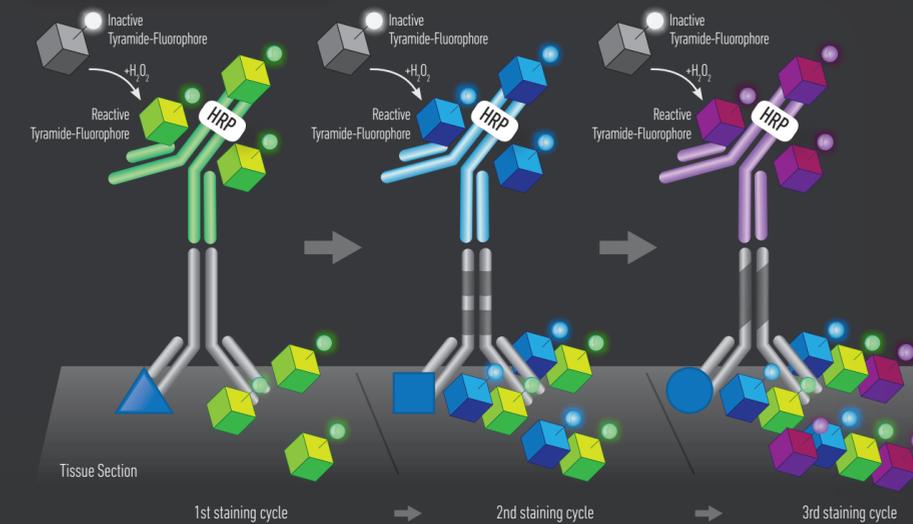
Without Amplification mIHC Process



Tyramide Signal Amplification mIHC

SEEING LIFE IN MULTICOLOR

With Amplification mIHC Process



mIHC allows multiple targets to be labeled. Traditional mIHC uses primary antibodies from different species. Tyramide signal amplification mIHC uses multiple rounds of staining and antibody removal.

Multiplex IHC for Immuno-oncology: Seeing Life in Multicolor

Sequential staining of different antigens with:

- Primary antibody
- HRP-conjugated secondary antibody
- Tyramide-conjugated fluorochromes

Tyramide labels adjacent to the antigens and binds covalently. Primary and secondary antibodies are removed by microwaving after each round of staining.

ADVANTAGES OF MULTIPLEX IHC

1. Multiplex IHC allows for visualization of multiple targets within a single tissue section, critical for limited samples.

2. Tissue architecture is preserved allowing for observation of spatial information and co-expression within the TME, unlike alternative multiplex approaches such as NGS, PCR, mass spectrometry, etc.

3. Fluorophore detection systems offer major advantages over chromogenic detection:

- Fluorophores have a wider dynamic range and larger linear range than chromogenic substrates, tyramide-based multiplexing enhances fluorescence signal enabling detection of low-level binding sites.
- DAPI (DNA/nuclear counterstain) is superior to hematoxylin, which can be obscured by other targets with chromogenic staining.
- Fluorescence signals can be overlaid and seen as single or multi-channel, allowing for intensity measurements for each target.