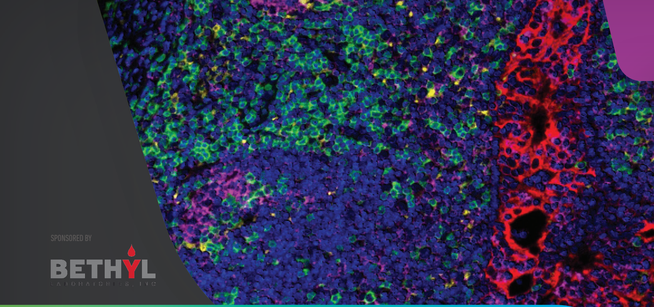

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Multiplex IHC: Making Discoveries Multicolor

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.

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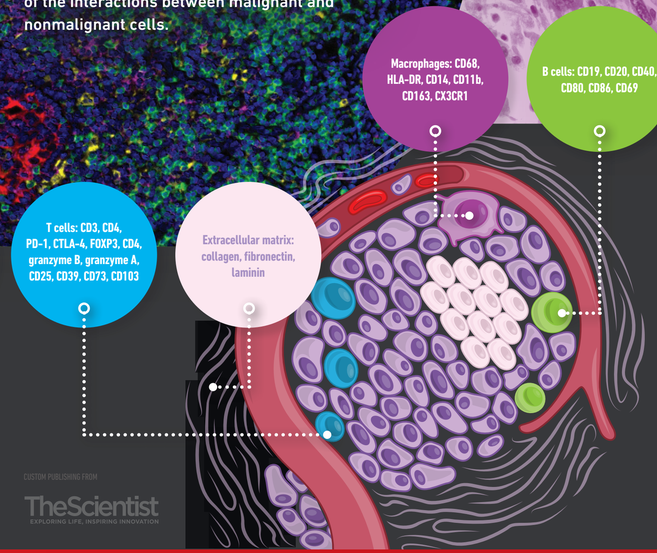
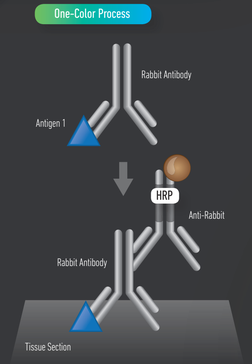
TME Expression Profiles

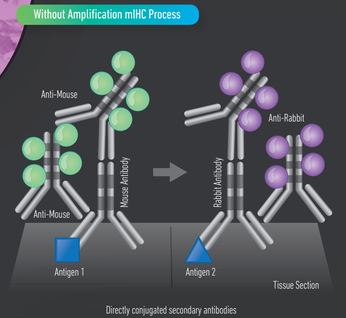
TME expression profiles guide understanding of the interactions between malignant and nonmalignant cells.



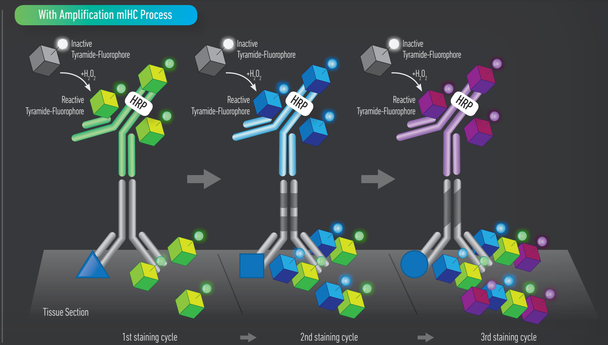
Traditional One-Color IHC | Traditional mIHC

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.





Tyramide Signal Amplification mIHC | SEEING LIFE IN MULTICOLOR



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.