BioTechniques In Focus

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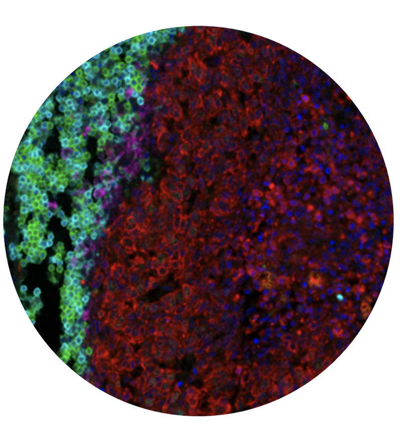
Multiplex immunofluorescence techniques explained

Multiplex immunohistochemistry-immunofluorescence (mIF) techniques enable the simultaneous detection of multiple proteins of interest in a single sample. This provides numerous benefits in the examination of different tissues, such as a tumor biopsy.



Top 5 benefits of mIF approaches:

1. Make the most of limited samples by visualizing multiple targets within a single tissue section.
2. Preserve tissue architecture.
3. Provide contextual, spatial data regarding colocalization and orientation of cells and proteins.
4. Capture microenvironment data.
5. Enable relative quantitation of target through assessment of fluorescent signal intensity.

Key techniques

Tyramide signal amplification

Working principle:

Deparaffinize sample in xylene and hydrate with ethanol and distilled water.

Conduct heat-induced epitope retrieval to facilitate antibody binding.

Tip
mIF is optimized for formalin-fixed paraffin-embedded tissues. Multiple rounds of heat-induced epitope retrieval can degrade other sample types.

Incubate with IHC-validated primary antibody.

Wash and incubate with horseradish-peroxidase (HRP) conjugated secondary antibody.

Wash and apply fluorophore-conjugated tyramide.

When tyramide interacts with HRP it forms covalent bonds with the tyrosine residues in or near the target protein.

Tip
Fluorophore pairings should be carefully considered for targets in the same cell type and especially in the same subcellular location. In these cases, use fluorophores with spectra that don't overlap.

Repeat these steps up to 8 times for different protein targets.

Counterstain with DAPI.

Tip
Pair high-intensity fluorophores with antibodies targeted to a low-abundance protein.

Image the panel and analyze the results.

Tip
The order of staining and imaging is important! For certain antibody–antigen pairs, stain intensity can vary based on its position in the workflow. Optimize your workflow to accommodate antibody–antigen pairs that are impacted by this.

Key Benefits

1. Compatible with any immunohistochemistry-validated antibodies.
2. Can detect low-abundance proteins.

Cyclic Immunofluorescence

Working principle:

Deparaffinize sample with xylene and rehydrate with water.

Conduct heat-induced epitope retrieval to facilitate antibody binding.

Capture background autofluorescence of the sample

Stain with fluorophore-conjugated primary antibodies for first two targets.

Acquire immunofluorescence.

Inactivate the fluorophore with:

Stain with fluorophore-conjugated antibodies against two more targets.

Acquire new immunofluorescence.

Repeat for up to 60 targets.

Once you have completed your rounds of Cyclic IF, conduct Hematoxylin and Eosin staining to allow for a conventional histopathology examination.

Compile image stacks for analysis.

Tip
Include a nuclear stain in each round of imaging to use for the alignment of image stacks.

Key Benefits

1. Can identify up to 60 targets.
2. Uses the same fluorophore in multiple rounds of staining, minimizing the need to use of uncommon fluorophores.

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