



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:



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.



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



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

Counterstain with DAPI.





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







