



10 TIPS FOR CHROMOGENIC MULTIPLEXING

Chromogenic multiplex staining needs careful planning and consideration to produce a multi-color, multi-target, permanent slide that is resistant to photo-bleaching, and perfect for brightfield imaging and downstream analysis.

CONSIDER COLOR COMBINATIONS

For colocalized markers, aim to use chromogens that will create a third color when combined.

CONSIDER USING LIGHTER CHROMOGEN COLORS

Lighter chromogen colors may be easier on the eyes to visualize especially when multiple chromogens are involved.

CONSIDER HIGH VS LOW EXPRESSION

Consider using a strong color chromogen for low expression, or low in quantity markers. Followed with a weaker color chromogen for high expression, or high in quantity markers.

OPTIMIZE THE CHROMOGEN SEQUENCE

DAB can overstain and occlude previously stained sites. Consider its suitable place in the assay.

CONSIDER THE RATE OF CHROMOGEN PRECIPITATION

Faster precipitating chromogens may be better suited for low expressed proteins. Consider slower precipitating chromogens for highly expressed proteins.

CONSIDER YOUR CHOICE OF PREFERRED COUNTERSTAIN

Ensure the counterstain provides appropriate contrast and does not interfere with chromogen interpretation.

CONSIDER SELECTING THE CHROMOGEN COLORS

Consider selecting the chromogen colors for spatially close targets first and then build the assay further.

CONSIDER THE COMPATIBILITY OF YOUR CHROMOGENS

Consider the compatibility of your chosen chromogens with your required mounting media and preferred dehydration methods.

TEST THE STABILITY OF DIFFERENT CHROMOGENS

Use chromogens with signals that remain strong earlier in the assay, and less robust signals later in the experiment.

CONSIDER THE ORDER OF YOUR MARKERS

Determine which antigens are robust or susceptible to degradation following multiple rounds of antigen retrieval.

[FOR MORE INFORMATION ABOUT MULTIPLEX STAINING, CLICK HERE.](#)

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