LOEWE®

Troubleshooting Guide Immuno-Fluorescence

Follow our immunofluorescence troubleshooting guide to quickly target the potential cause of a problem with your protocol and test solutions.

Identify the problem with your immunofluorescence staining from the options below:

1. Weak or No Staining
2. High Background
3. Non-specific Staining

1. Weak or No Staining

Incorrect light source/filter set:

- Ensure your microscope is equipped with the correct light source and filter set for the fluorophore you have chosen.

Gain/exposure is too low:

- Turn up the gain and/or increase the exposure time to ensure you are capturing any signal present.

Fluorescent tag bleached:

- Avoid over exposure of the slide to light sources for extended periods. Always store slides in the dark.

Cell/tissues are not fixed:

- Optimize the duration and temperature of fixation.

Over-extensive washing

- Be careful during washing steps. Too extensive washing might remove cells and/or antibodies from the slides

Not enough primary antibody:

- Use a higher concentration of antibody.
- Incubate longer.

The primary and secondary antibodies are incompatible:

- The secondary antibody should be raised against the host of the primary antibody. For example, if the primary antibody is derived from rabbit, use an Anti-rabbit secondary antibody

Slide storage issues:

- Samples should be imaged shortly after processing as the signal decreases over time. Store slides in the dark if needed.

Antibody Storage issues:

- Antibodies are stable during shipment at room temperature. However after arrival, storage should be at -20 °C. If the antibodies have not been stored as recommended a new vial is to be used instead.
- Freeze/thaw cycles are detrimental and can cause degradation. It is best to create aliquots of smaller amounts as soon as the product arrives at your location.
- If the secondary was not stored in the dark (when using immunofluorescence), a new vial will need to be used instead.

The antigen is not present in the tissues being tested:

- Run a positive control.
- If the antigen is present, but not abundantly, use an amplification step to maximize the signal.

Incubation time is too short:

- Increase the duration of incubation of the primary antibody with the sample.

2. High Background

Autofluorescence:

- Check to see if there is any fluorescence in an unstained section of the processed tissue. If there is, then this is autofluorescence in the tissue.

Tissue is too thick:

- Consider using thinner tissue dilutions.

Antibody Concentration is too high:

- Reduce the concentration of the primary and/or secondary antibody used.

Secondary is binding non-specifically:

- Run a secondary control without the primary. If there is staining, then change the secondary.

Insufficient washing:

- Proper washing of the tissue between steps is critical. Ensure you are following the protocol guidelines for wash steps.

3. Non-specific Staining

Spectral overlap:

- If imaging more than one fluorescent probe, the fluorophores may have excitation and emission spectra that overlap. Adjust your light sources and filters to pick up only one signal at a time. If this is not possible, choose new fluorophores that do not have spectral overlap.

Antibody Concentrations too high:

- Try reducing the concentration, and the incubation period.

Aggregates:

- Spin down secondary antibodies in a microcentrifuge to move aggregates to the bottom of the tube. Take from the top.

Need Help? Contact Us

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