FAQ – Why do I have no or only weak staining in IHC/IHC-P?

Primary and secondary antibodies are not compatible

Use a secondary antibody that is specific to the host species of the primary antibody. Make sure that the secondary antibody is compatible with the isotype of the primary antibody (e.g., IgG, IgM).

Antigen retrieval is required for epitope unmasking

Antigen retrieval (AGR) can unmask antibody epitopes in formaldehyde (PFA)-fixed tissue sections. It is essential for formalin fixed paraffin embedded (FFPE) tissues, but may also be required for certain targets in vibratome or cryostat sections. Staining outcomes are strongly influenced by AGR conditions including buffer composition, buffer pH, retrieval duration, and the chosen heat-induction method (e.g., microwave, vegetable steamer). The necessity and effectiveness of antigen retrieval also depend on the type and degree of fixation. Always consult the product-specific protocol for recommended AGR conditions. If needed, test different antigen retrieval methods and parameters to achieve optimal epitope exposure.

Use of inappropriate fixative or inappropriate fixation procedure

SYSY Antibodies reference tissues are fixed in formalin for 24 hours. Deviations from this—such as prolonged fixation, insufficient fixation, or the use of alternative fixatives—can significantly affect antibody performance. Optimize your staining protocol by testing different antigen retrieval methods and conditions to effectively unmask the epitope. Whenever possible, standardize your fixation procedure to ensure consistent and reproducible results.

Protein is not present in the tissue of interest or is only expressed at low levels

Run a positive control in your staining procedure. A positive result from a tissue known to express the protein of interest will verify that your staining protocol is working.

When the protein of interest is only expressed at low levels, utilize a higher sensitivity staining system.

Use of inappropriate dehydration protocol or mounting medium

DAB-stained slides may be dehydrated, cleared, and mounted with an organic mounting medium as described in our **SYSY Antibodies Reference Protocols**. When using alternative HRP substrates or AP substrates, please consult the respective manufacturer's instructions regarding alcohol tolerance and mounting-medium compatibility (aqueous or non-aqueous) to prevent loss of chromogenic signal.

Fluorescently stained slides can be air-dried or mounted while still wet. Please refer to the manufacturer's recommendations to ensure that the chosen mounting medium is compatible with the fluorophore.