FAQ – Why do I have no or only weak staining?

Primary and Secondary Antibodies are not Compatible

Use a secondary antibody that was raised against the species in which the primary antibody was made. Make sure that the secondary antibody is compatible with the isotype of the primary antibody (e.g. IgG, IgM).

Antigen Retrieval was not Successful

IHC-P staining results are extremely influenced by antigen retrieval conditions including buffer composition, buffer pH, antigen retrieval time, and method (e.g. microwave, vegetable heat steamer). The need for antigen retrieval is furthermore dependent on the type and extent of fixation. Try different antigen retrieval methods and conditions to unmask the epitope.

Use of Inappropriate Fixative or Inappropriate Fixation Procedure

SYSY reference tissues are fixed in formalin for 24 hours. Prolonged fixation, under-fixation, and use of alternative fixatives have a major impact on antibody performance. Optimize the staining protocol by trying different antigen retrieval methods and conditions to unmask the epitope. Standardize your fixation procedure.

The 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 can be dehydrated, cleared, and mounted with an organic mounting medium as delineated in our SYSY reference protocol. When using other enzyme substrates for HRP-detection or when using AP-detection substrates, you must refer to the manufacturer's instructions for alcohol compatibility and mounting medium compatibility (aqueous or non-aqueous) to avoid loss of chromogenic signal.