FAQ – Why do I have high background or non-specific staining?

Primary / Secondary Antibody Concentration may be too High

The recommended primary and secondary antibody concentrations provide good staining results in SYSY reference protocols. However, IHC-P staining results are extremely influenced by antigen retrieval conditions (buffer composition, pH, time, method), primary and secondary antibody incubation times, blocking solutions, secondary systems, and tissue types. Thus, optimal concentration for primary and secondary must be determined individually. SYSY recommends to titrate primary and secondary antibodies for each tissue type.

Endogenous Enzyme Interference when using Chromogenic Detection

Tissues such as kidney or liver contain high amounts of endogenous peroxidases which can produce false positive staining in HRP-based antibody detection. Use 3% H_2O_2 to block endogenous peroxidase activity. Certain antigens can be destroyed by high concentrations of hydrogen peroxidase. In this case, perform the H_2O_2 block after primary antibody incubation.

Endogenous AP is, for example, found in intestine, kidney, and lymphoid tissues and can be blocked with 1 mM Levamisole when performing AP-based antibody detection.

SYSY recommends testing for endogenous enzyme activity by omitting the primary and secondary antibodies (and ABC system when applicable) in the staining protocol.

Endogenous Biotin when using the ABC Method

Certain tissues (e.g. kidney, liver) contain high amounts of endogenous biotin. Especially when using Tris-EDTA or EDTA-based antigen retrieval buffers, the ABC-method can lead to high background and / or false positive staining. SYSY thus recommends using an Avidin-Biotin blocking step. If the background problem persists, consider trying a polymer-based detection system instead of the ABC-method

SYSY recommends testing for endogenous biotin when using the ABC method by incubating only the avidin-biotin-complex and omitting the primary and the biotinylated secondary antibody in the staining protocol.

Sections have Dried Out

Do not let sections dry out. Use a humified chamber for antibody incubation.

The Secondary Antibody causes Background Staining

Monoclonal HistoSure antibodies against mouse proteins are generated in rat instead of mouse to avoid classic “mouse-on-mouse” problems. However, anti-rat secondary antibodies need to be mouse-adsorbed when used on mouse tissues.

Other secondary antibodies can also produce unexpected unspecific staining. SYSY recommends testing for putative cross-reactivity of the secondary antibody by omitting the primary antibody in the staining protocol. Perform Lot-to-Lot comparisions for your secondary reagents as well!

Blocking is Insufficient

SYSY uses serum-free protein block and antibody diluent (which has an additional blocking effect) from Agilent in reference protocols. Optimize blocking conditions when using alternative blocking agents. When using normal serum for blocking, use normal serum from the host of the secondary antibody.

Tissue Sections too Thick

Cut tissue sections thinner. FFPE tissue sections should be 2.5 - 5 µM.