Multiple Antigen Labeling Guide for the ImmPRESS™ Excel Amplified HRP Polymer Staining Kit

Introduction

The ImmPRESS™ Excel Amplified HRP (Peroxidase) Polymer Staining Kit is a peroxidase-based, non-biotin amplification system that produces crisp, highly sensitive, specific staining with low background. These characteristics are important in the detection of low copy antigens or primary antibodies used at high dilution due to their availability or cost. The ImmPRESS™ Excel Kit staining protocol is short, the reagents are provided as ready-to-use or easily diluted stocks, and the results are reproducible.

The ImmPRESS™ Excel kits can be used for labeling multiple antigens in the same tissue section. However, as with any multiple antigen labeling experiment, deletion controls should be run to verify the specificity of each label. When planning and interpreting the deletion controls, the components of this kit should be taken into account.

A) Amplifier Antibody:

The Amplifier Antibody in both the ImmPRESS™ Excel Anti-Mouse Ig Kit and the ImmPRESS™ Excel Anti-Rabbit Ig Kit is made in goat. When ImmPRESS™ Excel kits are used for multiple labeling, even if the primary antibodies are made in rabbit and mouse, care must be taken that the ImmPRESS™ Polymer Detection Reagent (an anti-goat IgG) used in the second staining procedure does not bind to the Amplifier Antibody (made in goat) used in the first staining procedure, or that the ImmPRESS™ Polymer Detection Reagent used in the first staining procedure does not bind the Amplifier Antibody used in the second staining procedure.

This potential for "cross-reaction" is analogous to any multiple antigen labeling application in which all the primary antibodies are produced in the same species. This cross-reaction can be prevented by two methods:

a) The reaction product of the first chromogen can provide an effective "covering" or "sheltering" of all the reagents used in the preceding staining protocol, eliminating cross-reactivity with subsequent steps. Titering the primary antibody to optimize substrate sheltering is required.

b) A heat denaturing step (HIER, heat induced antigen retrieval) used before each subsequent labeling protocol denatures most antibody reagents preventing most antibody/antibody cross-reactions. An effective procedure uses either of Vector's Antigen Unmasking Solutions (citrate based or high pH based) with a high temperature treatment, such as a pressure cooker, between sequential labeling protocols.

B) ImmPRESS™ Excel Polymer Detection Reagent:

The detection enzyme used in the ImmPRESS™ Excel Kits is horseradish peroxidase. When using the same detection enzyme for both labeling protocols, the enzyme from the first labeling protocol must be completely inactivated or sheltered, in order to prevent artifactual co-staining.

Fortunately, the methods used to prevent "cross-reaction" for the Amplifier Antibody – (1) "sheltering" by the first substrate deposition or (2) removing the antibody reagents in the first system using HIER - will also address this concern.

Please note: A heat denaturing step does not inactivate endogenous peroxidases. If endogenous peroxidase activity is present in the tissue, an endogenous enzyme blocking reagent such as BLOXALL™ Blocking Solution should be used at the start of the staining procedure.

C) ImmPACT™ DAB EqV Chromogen:

ImmPACT™ DAB EqV substrate is included as the HRP substrate chromogen in the ImmPRESS™ Excel Kit. The reaction product of this substrate is heat stable. This is important if a heat denaturing step (described above) is used before subsequent labeling protocols.

Additional HRP chromogen(s) will be required for additional color(s). The choice depends on several considerations: sensitivity, color contrast, type of microscopy, and heat resistance.

Charts showing the relative substrate sensitivities, substrate compatibilities, staining sequence, heat resistance and counterstain compatibility, as well as images of each substrate, are available on the Vector Labs website (www.vectorlabs.com).

Suggested multiple labeling protocols, controls, trouble-shooting information, and images are available on the Vector Labs website or in our booklet; Discovery through Color - A Guide to Multiple Antigen Labeling.

Of course, all staining protocols need to be optimized for a particular application. The wash time listed in the protocols, especially after the ImmPRESS™ Reagent, should be followed and can be lengthened by several minutes. Also, primary antibody titers should be re-optimized for multiple labeling applications. The appropriate deletion controls should always be run to allow for the accurate interpretation of the results.