WHITEPAPER

Reduction of background staining in mouse tissues with Rodent Block-M™

BIOCARE MEDICAL

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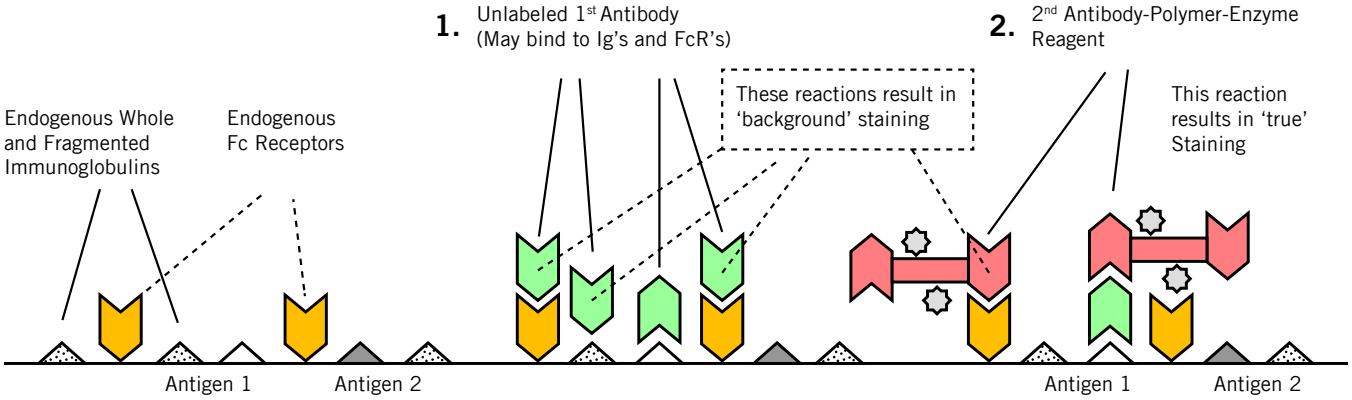
Just as various target protein (epitopes) exist 'naturally' within specimen material, so do (intact) immunoglobulin (Ig) molecules, 'portions' of Ig's (known as Fc fragments) and Fc receptors, which will be referred in the remainder of this document as 'sticky proteins' (SPs). Since primary antibodies and many commonly-employed detection systems incorporate anti-rodent – i.e. mouse and rat – primary and secondary antibodies, these reagents can undoubtedly bind to SPs. Such staining is not, however, 'discreet' or specific, – i.e. limited to the cytoplasm, membrane and/or nucleus of particular cells – rather, it is observed as 'background', which maybe so significant as to make interpretation of desired staining very difficult.

Since the detection of antibodies produced in mice and rats is occasionally performed on specimens obtained from rodent models of human disease, it is useful to understand the expected (but undesirable) reaction between SPs and primary/secondary antibodies. Figure 1 demonstrates how such reagents can bind to specimens.

Figure 1

Without Blocking – Applied primary and secondary antibodies (incorporated into detection reagents) can bind to whole immunoglobulins, Fc fragments, and Fc receptors within specimen material



Is SP Blocking Required?

Although SP 'blocking' procedures are not required, staining is nearly always improved when such steps are included, depending upon the nature of the target-protein and the specificity/sensitivity of the detection system. The best way to determine if such blocking is necessary is to perform 'side-by-side' staining reactions; if staining is improved, then, clearly, blocking should be performed on an ongoing basis.

Practical Application of These Principles

So, how does one incorporate the blocking procedures outlined above into a given IHC staining protocol, manual or automated? That's easy! Rodent Block-M™*, designed for blocking endogenous mouse IgG and non-specific background in mouse tissue and red blood cells, is applied prior to application of the primary antibody solution, and, if necessary, prior to application of the secondary antibody-polymer-enzyme reagent (e.g. Biocare's Rat-On-Mouse detection**, Mouse-on-Mouse***, or Rabbit-on-Rodent****).The diagram shown in Figure 2 below is intended to demonstrate the mode of action of SP blocking agents. A sample protocol that incorporates these additional steps is shown in Figure 3 below.

If your laboratory is interested in learning more about these or other related products, please contact Biocare's Technical Support department (800-799-9499, option 3). You might also be interested in reviewing the companion document in this series, entitled "Reduction of background staining in rat tissues with Rodent Block-R™".

Figure 2

With Blocking – Blocking agents prevent primary and secondary antibodies (incorporated into detection reagents) from binding to proteins within specimen material

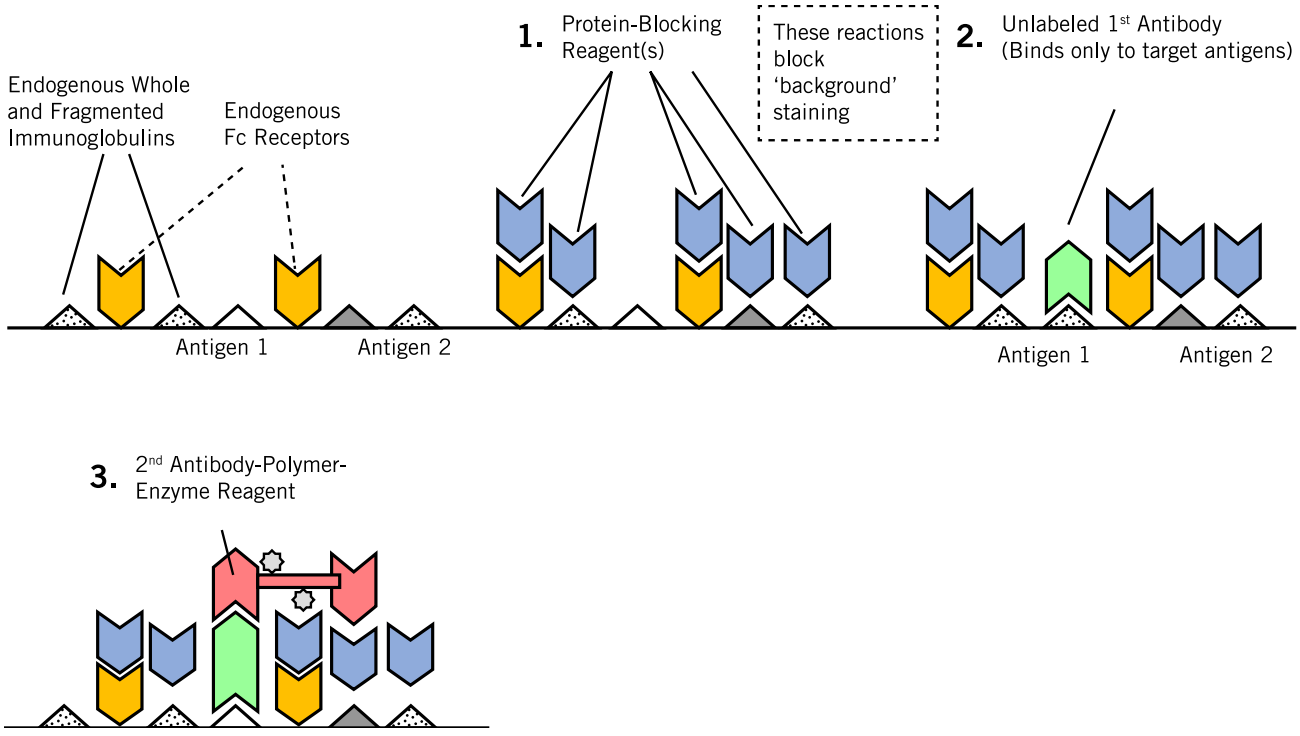


Figure 3

Sample protocol employing Rodent Block-M™ on mouse tissue

1. Deparaffinize slide-mounted tissue sections by established procedures;
2. Perform heat-induced or digestion-induced antigen retrieval by established procedures;
3. If necessary/desired, perform endogenous-enzyme 'quenching' by established procedures';
4. If necessary/desired, perform endogenous-protein/Ig/Fc-receptor 'blocking' procedures by applying Rodent Block-M™*to all slides (tissues) – incubate for 15 to 30 minutes at room temperature (RT);
5. Rinse slides thoroughly with Tris-Buffered Saline (TBS);
6. Apply primary antibody solution – incubate as desired;
7. Rinse slides thoroughly with TBS;
8. (Optional: If necessary/desired, perform endogenous-protein/Ig/Fc-receptor 'blocking' procedures by applying Rodent Block-M™ to all slides (tissues) – incubate for 10 to 20 minutes at room temperature RT);
9. Rinse slides thoroughly with TBS;
10. Apply secondary antibody/polymer/enzyme reagent – incubate for 15 to 30 minutes at RT
11. Rinse slides thoroughly with TBS;
12. Complete remainder of protocol as necessary.

*Refers to Biocare's Rodent Block-M™ – Product code: RBM961
**Refers to Biocare's Rat-On-Mouse-HRP™ and Rat-On-Mouse-AP™ detection reagents – Product codes: RT517 and RT518, respectively.
***Refers to Biocare's Mouse-On-Mouse-HRP™ and Mouse-On-Mouse-AP™ detection reagents – Product codes: MM620 and MM624, respectively.
****Refers to Biocare's Rabbit-On-Rodent-HRP™ and Rabbit-On-Rodent-AP™ detection reagents- Product codes: RMR622 and RMR625, respectively.