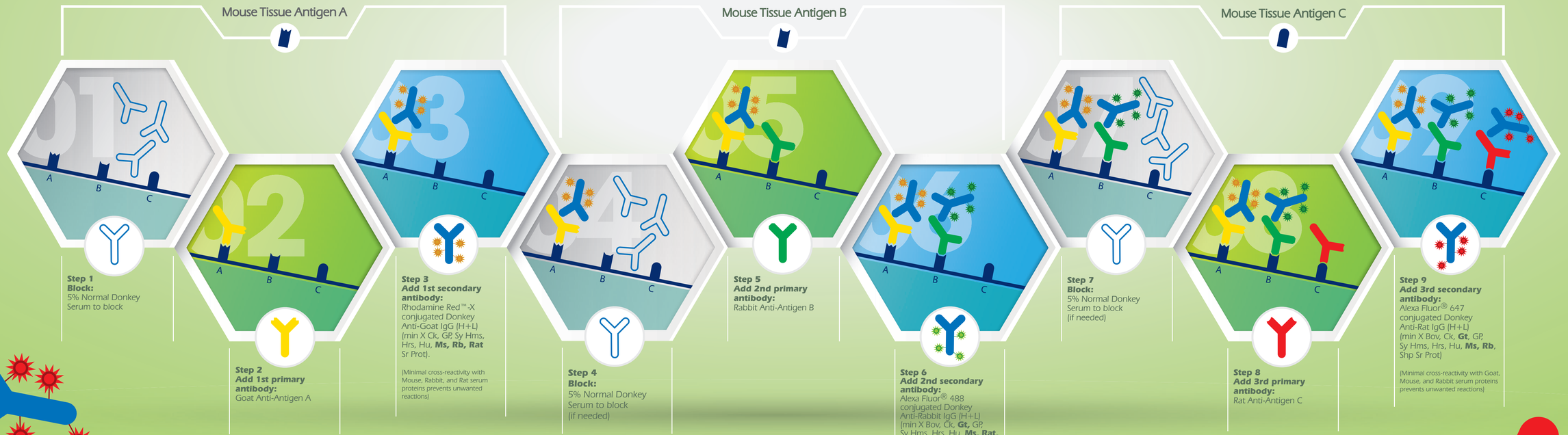
Multiple Labeling

Our guide to multiple labeling with Secondary Antibodies

Jackson ImmunoResearch Europe Ltd

Jackson ImmunoResearch provides secondary antibodies with minimal cross-reactivity for multiple labeling. These are designated "ML" in our catalog and online. An example multiple-labeling protocol using these reagents is shown in the following 9 Step example.



Step 1
Block:
5% Normal Donkey Serum to block

Step 2
Add 1st primary antibody:
Goat Anti-Antigen A

Step 3
Add 1st secondary antibody:
Rhodamine Red™-X conjugated Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, **Ms, Rb, Rat** Sr Prot).

(Minimal cross-reactivity with Mouse, Rabbit, and Rat serum proteins prevents unwanted reactions)

Step 4
Block:
5% Normal Donkey Serum to block (if needed)

Step 5
Add 2nd primary antibody:
Rabbit Anti-Antigen B

Step 6
Add 2nd secondary antibody:
Alexa Fluor® 488 conjugated Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, **Gt,** GP, Sy Hms, Hrs, Hu, **Ms, Rat,** Shp Sr Prot)

(Minimal cross-reactivity with Goat, Mouse, and Rat serum proteins prevents unwanted reactions)

Step 7
Block:
5% Normal Donkey Serum to block (if needed)

Step 8
Add 3rd primary antibody:
Rat Anti-Antigen C

Step 9
Add 3rd secondary antibody:
Alexa Fluor® 647 conjugated Donkey Anti-Rat IgG (H+L) (min X Bov, Ck, **Gt**, GP, Sy Hms, Hrs, Hu, **Ms, Rb**, Shp Sr Prot)

(Minimal cross-reactivity with Goat, Mouse, and Rabbit serum proteins prevents unwanted reactions)

Selection of antibodies for simultaneous detection of more than one antigen depends on at least two important criteria:

1. Availability of secondary antibodies that do not recognize (a) one another (are derived from the same host species), (b) other primary antibodies used in the assay system, (c) immunoglobulins from other species present in the assay system, or (d) endogenous immunoglobulins present in the tissues or cells under investigation.
2. Use of probes (enzyme-reaction products, fluorophores, or electron-dense particles) that are well resolved.

Notes: Optimize individual primary/secondary labeling protocols prior to attempting multiple labeling. Wash thoroughly after each step, including after blocking at step 1. With heavy or persistent background further blocking may be required at Steps 4 and 7. Do not dilute any antibody with normal serum or mix antibodies together to save time, which may result in immune complex formation which could increase background. In this example, the secondary antibodies used in Steps 3, 6 and 9 do not recognize each other since they are all made in donkey. They have been solid-phase adsorbed so that they do not recognize the other primary antibodies used in Steps 2, 5 and 8. Also, they do not react with endogenous mouse Ig, which may be present in the mouse tissue.

For a review of multi-color immunofluorescence labeling with confocal microscopy see Brelje, Wessendorf, and Sorenson, "Multi-color laser scanning confocal immunofluorescence microscopy: Practical application and limitations." In Cell Biological Applications of Confocal Microscopy (Methods in Cell Biology, **vol. 38**), Ed. B. Matsumoto, Orlando, FL: Academic Press, Inc. 1993, pp. 98-181.

Ordering Information

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