Jackson ImmunoResearch LABORATORIES, INC.

EXAMPLE C

Use of unconjugated Fab fragments for blocking after the first secondary antibody step

Read more about Fab fragments at: jacksonimmuno.com

Detection of two unlabeled primary antibodies from the same host species

Example C shows one of the possible protocols used for double labeling two unconjugated primary antibodies from the same host species. For more protocols visit: www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary.

The success of these experimental designs will require some empirical manipulations. Optimizing reagent concentrations in each step or switching the labeling sequence of the two antigens may influence the outcome.

- Labeling the less abundant primary antibody first increases blocking efficiency.
- Blocking with an appropriate normal serum helps to reduce background.
- To avoid displacement of the Fab antibody by the labeled secondary antibody, a light post-fixation with glutaraldehyde may be used, provided that it does not affect antigenicity of the target proteins.

EXAMPLE C

Detection of two unlabeled primary antibodies from the same host species

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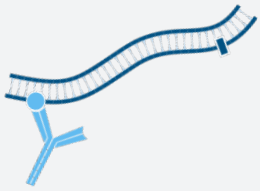
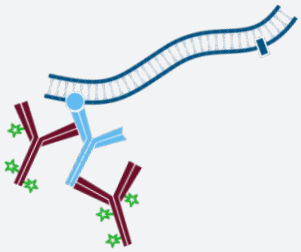
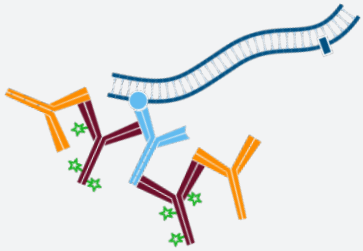


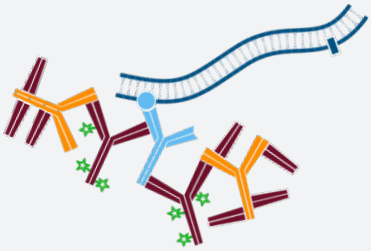
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.



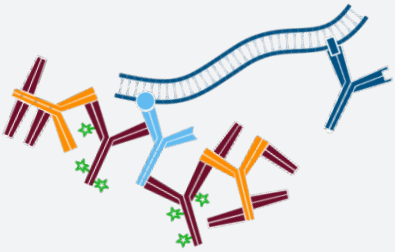
2. Incubate with conjugated secondary antibody, in this example Alexa Fluor® 488-Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot). Wash.



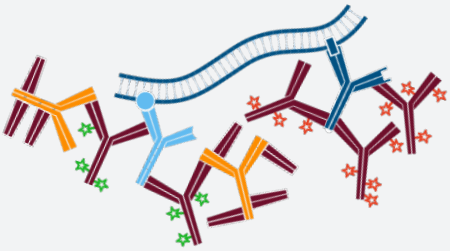
3. Incubate with normal serum from the same host species as the primary antibodies, in this example normal rabbit serum. The purpose of this step is to saturate open binding sites on the first secondary antibody with IgG so that they cannot capture the second primary antibody. Wash.



4. Incubate with an excess of unconjugated Fab antibody against the host species of the primary antibodies, in this example Fab Goat Anti-Rabbit IgG (H+L). The host species of the Fab antibody should be the same as the host species of the conjugated secondary antibody. This step covers the rabbit IgG so that the second secondary antibody will not bind to it. Wash.

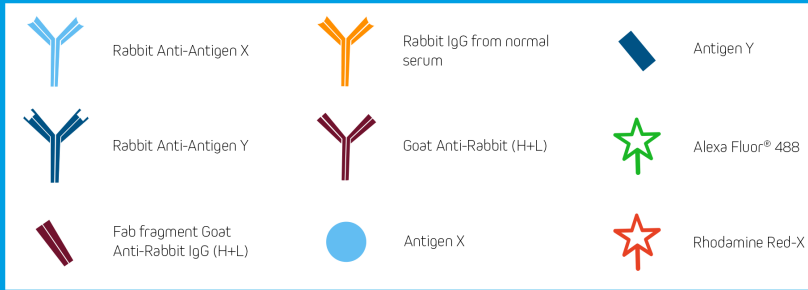


5. Incubate with the second primary antibody, in this example Rabbit Anti-Antigen Y. Wash.



6. Incubate with the same secondary antibody as used in step 2, conjugated to a different probe, in this example Rhodamine Red™-X-Goat Anti-Rabbit IgG (H+L) (min X Hu, Ms, Rat Sr Prot). Wash.

Key



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