Jackson ImmunoResearch LABORATORIES, INC.

EXAMPLE A

Use of conjugated Fab fragments for labeling and blocking

Read more about Fab fragments at: jacksonimmuno.com

Detection of two unlabeled primary antibodies from the same host species

Example A 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.

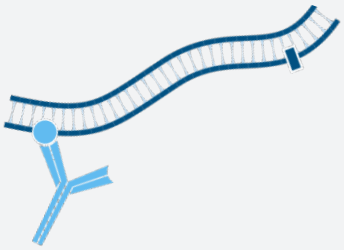
The success of these experimental designs will require some empirical manipulations. Optimizing reagent concentrations in each step or switching the labeling sequence of the two antigens may influence the outcome.

- 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.

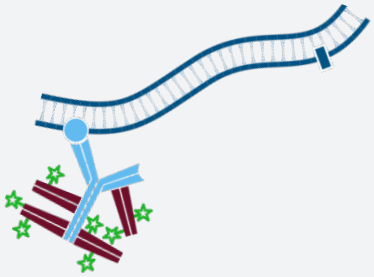
Important note: The monovalent Fab fragments have not been adsorbed to remove cross-reactivities to other species. If the experimental sample contains endogenous immunoglobulins Example C should be used. Example A or B could introduce background.

EXAMPLE A

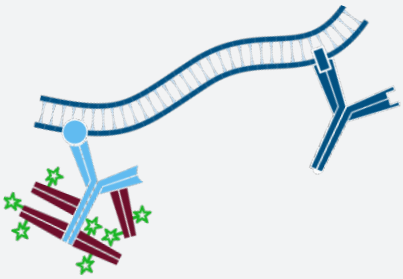
Detection of two unlabeled primary antibodies from the same host species



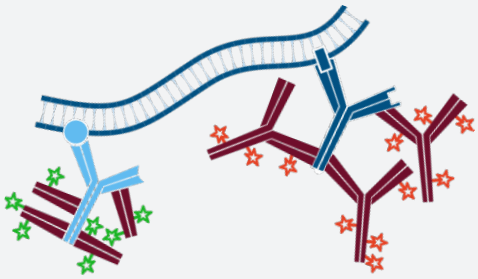
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.



2. Incubate with excess conjugated secondary antibody, in this example Alexa Fluor® 488-Fab fragment Goat Anti-Rabbit IgG (H+L). Wash.



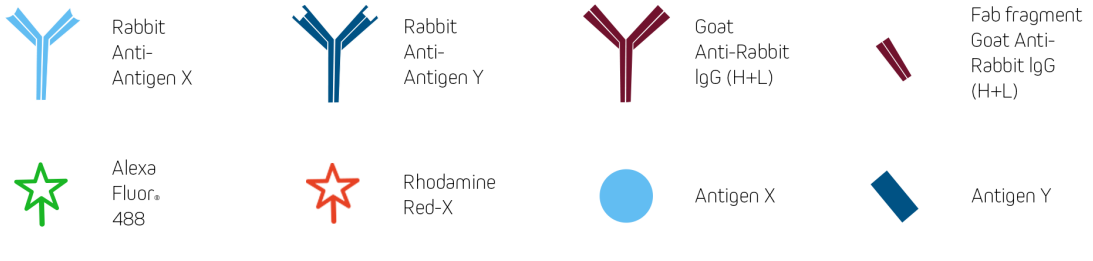
3. Incubate with the second primary antibody, Rabbit Anti-Antigen Y.



4. Incubate with a second conjugated secondary antibody, in this example Rhodamine Red™-X-Goat Anti-Rabbit IgG (H+L). Wash.

Application notes: (1) Monovalent Fab fragments have not been adsorbed against other species, so they may cross-react with endogenous Ig. Use Example C to avoid detection of endogenous Ig.
(2) Example A may require a high concentration of conjugated Fab to saturate the first primary antibody. If this results in unacceptable background, try a lower concentration of the conjugated Fab, followed by further blocking with unconjugated Fab.

Key



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