

## EXAMPLE B

## Use of unconjugated Fab fragments to cover the first primary antibody, presenting it as a different species

Read more about Fab fragments at: jacksonimmuno.com

## Detection of two unlabeled primary antibodies from the same host species

Example B shows one of the possible protocols used for double labeling two unconjugated primary antibodies from the same host species. For more protocols visit: <a href="https://www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary">www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary</a>.

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 <u>Example C</u> should be used. Example A or B could introduce background.



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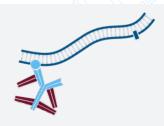
## **EXAMPLE B**

Detection of two unlabeled primary antibodies from the same host species

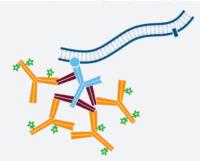




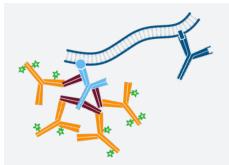
1. After blocking with normal serum, incubate with the first primary antibody, in this example Rabbit Anti-Antigen X. Wash.



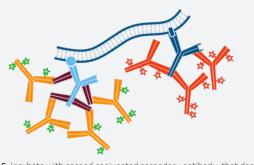
2. Incubate with an excess of unconjugated Fab antibody against the host species of the primary antibody, in this example unconjugated Fab fragment Goat Anti-Rabbit IgG (H+L). This presents the rabbit IgG as goat Fab. Wash.



3. Incubate with conjugated tertiary antibody directed against the host species of the Fab antibody. The tertiary antibody must not recognize the host species of either the primary antibodies or the second secondary antibody. This example used Alexa Fluor  ${\bf @}$ 488-Mouse Anti-Goat IgG (H+L) (min X Ms, Hu, Rb Sr Prot). Wash.



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



5. Incubate with second conjugated secondary antibody, that does not recognize the host species of either the Fab antibody used in step 2 or the tertiary antibody used in step 3. In this example,  $Rhodamine\ Red^{\text{\tiny{TM}}}\text{-}X\text{-}Mouse\ Anti-Rabbit\ lgG}\ (H+L)\ (min\ X\ Hu,\ \textbf{Gt},$ Ms, Shp Sr Prot) was used. Wash.

**Application note:** Monovalent Fab fragments have not been adsorbed against other species, so they may cross-react with endogenous Ig. Use

