

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: 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.

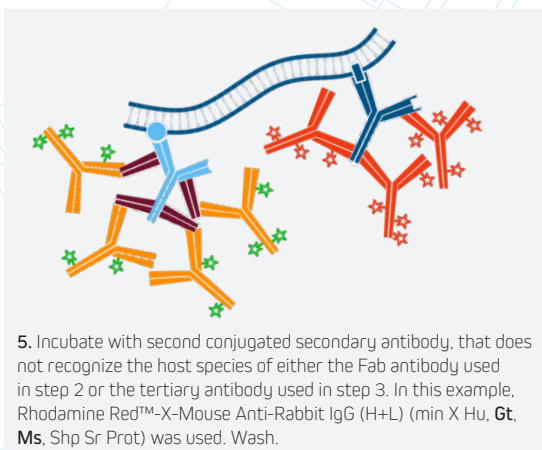
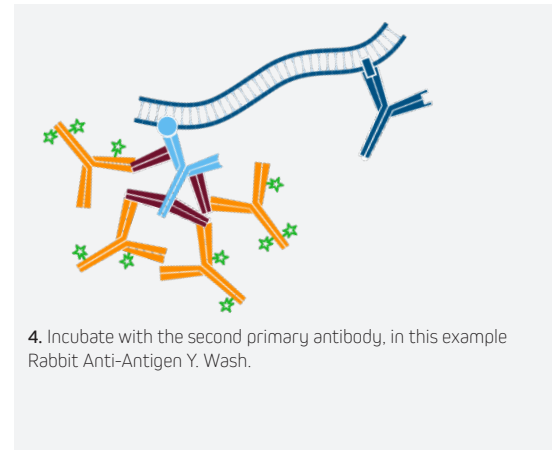
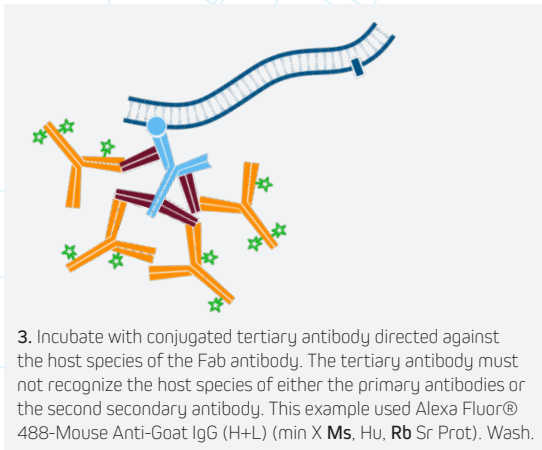
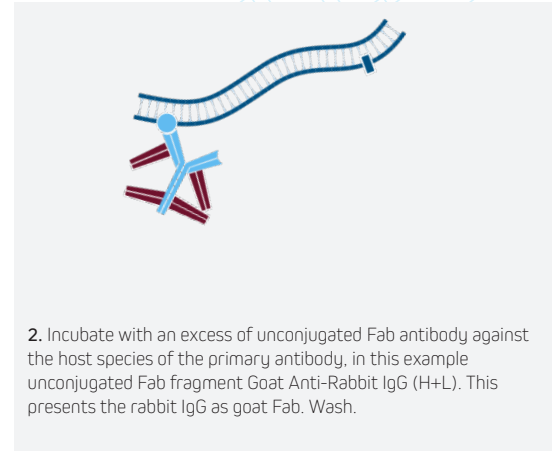
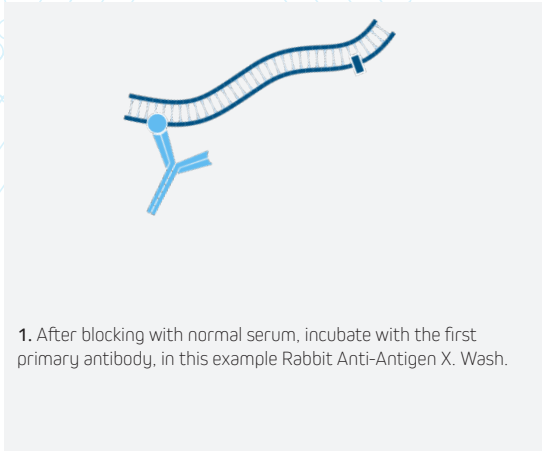
Important note: The monovalent Fab fragments have not been adsorbed to remove cross-reactivities to other species. If the experimental sample contains endogenous immunoglobulins [Example C](#) should be used. Example A or B could introduce background.



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Detection of two unlabeled primary antibodies from the same host species



Application note: 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.

Key

	Rabbit Anti-Antigen X		Mouse Anti-Goat IgG (H+L) (min X Ms, Hu, Rb Sr Prot)		Antigen Y
	Rabbit Anti-Antigen Y		Mouse Anti-Rabbit (H+L) (min X Hu, Gt, M, Shp Sr Prot)		Alexa Fluor® 488
	Fab fragment Goat Anti-Rabbit IgG (H+L)		Antigen X		Rhodamine Red-X