

## EXAMPLE D

# Use of unconjugated Fab fragments for detection of one unlabeled and one or more labeled primary antibodies

Read more about Fab fragments at:  
[jacksonimmuno.com](http://jacksonimmuno.com)

## Detection of one unlabeled and one or more labeled primary antibodies from the same host species

Example D illustrates a multiple labeling protocol that includes a directly labeled and an unlabeled primary antibody. It is advisable to incubate the less abundant primary first. In Example D, the directly labeled primary antibody is incubated first, then blocked with Fab fragments prior to applying the unlabeled primary antibody.

For more protocols visit: [www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary](http://www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary).

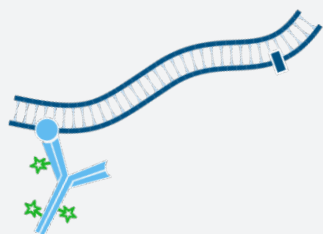
If the unlabeled primary antibody is incubated first, double labeling can be achieved without using Fab fragments. Following incubation with the labeled secondary antibody, normal serum is used to block open binding arms of the secondary, preventing capture of the labeled primary. (See Example E).



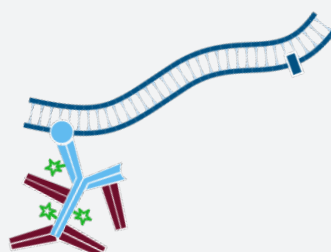
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## EXAMPLE D

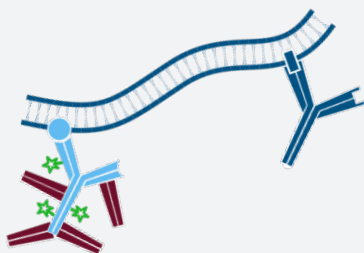
Detection of one unlabeled and one or more labeled primary antibodies from the same host species



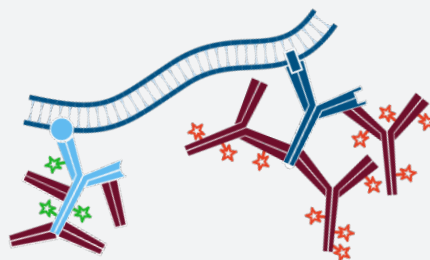
1. After blocking with normal serum, incubate with conjugated primary antibody, in this example Alexa Fluor® 488-Rabbit Anti-Antigen X. Wash.



2. Incubate with an excess of unconjugated Fab Goat Anti-Rabbit IgG (H+L). Wash.



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



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

### Key



Rabbit  
Anti-  
Antigen X



Rabbit  
Anti-  
Antigen Y



Goat  
Anti-Rabbit  
IgG (H+L)



Fab fragment  
Goat Anti-  
Rabbit IgG  
(H+L)



Alexa  
Fluor®  
488



Rhodamine  
Red-X



Antigen X



Antigen Y

