Jackson ImmunoResearch LABORATORIES, INC.

BLOCKING

Use of unconjugated Fab fragments to block endogenous immunoglobulins and avoid off target signal

Read more about Fab fragments at: jacksonimmuno.com

Blocking Endogenous Immunoglobulins With Fab Fragments

Background staining may be observed if a labeled secondary antibody is not adsorbed to minimize recognition of endogenous tissue Ig. When a primary antibody is the same species as the tissue under study (e.g. mouse primary used on mouse tissue), blocking endogenous Ig suppresses the off-target signal.
For more protocols visit: www.jacksonimmuno.com/technical/products/protocols/double-labeling-same-species-primary.

To block endogenous immunoglobulins on cells or tissue sections, incubate with an excess (20-40 µg/ml) of unconjugated Fab antibody just after blocking with 5% normal serum. Blocking efficiency can be confirmed by eliminating the primary antibody from the protocol and incubating with labelled secondary antibody. It may be necessary to increase the concentration of Fab antibody up to 100 µg/ml to suppress signal from high levels of endogenous IgG.

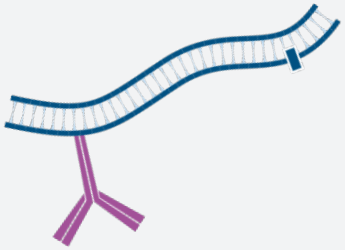
To avoid displacement of the Fab antibody by the labelled secondary antibody, a light post-fixation with glutaraldehyde may be necessary, provided that it does not affect antigenicity of the target proteins. Fab antibodies are not as effective for blocking immunoglobulins in Western blotting or ELISA applications. For more information see our blog article: https://www.jacksonimmuno.com/secondary-antibody-resource/technical-tips/controls-diluents-blocking.

bsi. ISO 9001:2015 Quality Management

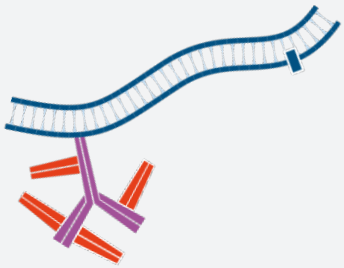
Jackson ImmunoResearch Laboratories Inc. is certified by BSI to ISO 9001:2015 under certificate number FM 545248.

BLOCKING

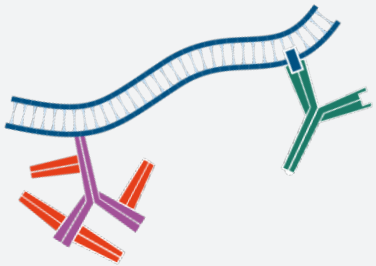
Blocking Endogenous Immunoglobulins With Fab Fragments



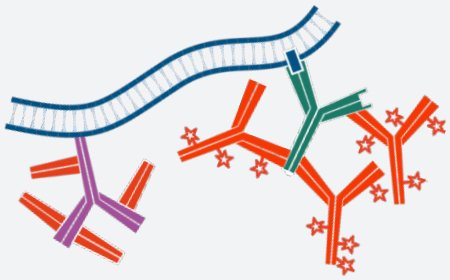
1. Samples may express endogenous immunoglobulins, in this example mouse IgG.



2. After blocking with normal serum, incubate with an excess of unconjugated Fab antibody, in this example Fab fragment Goat Anti-Mouse IgG (H+L). Wash.



3. Incubate with primary antibody, in this example Mouse Anti-Antigen X. Wash.



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

Key

Mouse IgG

Mouse Anti-Antigen X

Goat Anti-Mouse IgG (H+L)

Antigen X

Rhodamine Red-X

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

+1 800-367-5296 cuserv@jacksonimmuno.com www.jacksonimmuno.com