Jackson ImmunoResearch LABORATORIES INC.

A GUIDE TO

Blocking & Control Reagents

- Reduce background
- Troubleshoot off-target signal
- Facilitate analysis
- Improve reproducibility

Optimize Your Experiments

from Jackson ImmunoResearch

Scan the code to access more information online

When developing an immunotechnique, it is important to consider result analysis. The addition of the correct blocking reagents and experimental controls can improve assay performance, interpretation and ultimately the presentation of data for publication.

Use this guide to learn more about the selection of appropriate control, diluent and blocking reagents in a range of immunotechniques

A GUIDE TO

Blocking & Control Reagents

Jackson ImmunoResearch LABORATORIES INC.

Whatever your application, JIR can help you troubleshoot. Read our brief introduction below to see how JIR immunoreagents can help your assay.

Flow Cytometry

Problem	Solution	Indicated product
Background from antibodies binding Fc receptors	Block Fc receptors.	Normal serum from the host of the labeled antibody
	Use $F(ab')_2$ format secondary antibody to avoid entrapment by Fc receptors.	$F(ab')_2$ secondary antibodies
Confirmation that primary antibody binding is due to antigen specificity	Use an isotype negative control (non-specific IgG from the same species as the primary antibody) to demonstrate specific binding of the primary antibody.	ChromPure™ purified proteins
Confirmation that secondary antibody does not contribute to off-target signal	Use an isotype negative control (conjugated non-specific IgG from the same species as the secondary antibody) to demonstrate specific binding of the secondary antibody.	ChromPure™ purified proteins
Short on time and no directly conjugated primary available	Fab label your primary antibodies prior to incubation with sample.	FabuLight™

Immunostaining

Problem	Solution	Indicated Product
Confirmation that primary antibody binding is due to antigen specificity	Use an isotype negative control (non-specific IgG from the same species as the primary antibody) to demonstrate specific binding of the primary antibody.	ChromPure™ proteins
Background (general)	Block endogenous binding sites which may interact with experimental reagents.	Normal serum from the host of the labeled antibody.
	Dilute antibodies in buffer without carrier proteins. Centrifuge working dilution to remove aggregates.	Correct diluent and preparation, e.g. PBS/ Tween 20
Background (homologous Ig recognition)	Use a cross-adsorbed secondary antibody with minimal cross-reactivity to the cell or tissue species being analyzed.	Min X secondary antibodies
	Block endogenous immunoglobulins.	Fab fragments
Multiple labeling of primary antibodies from same host species (e.g. mouse on mouse)	Utilize Fab fragments in suggested protocols to accomplish multiple labeling (see online).	Fab fragments
	Immunolabel primary antibody prior to incubation.	FabuLight™
Endogenous enzymes	Inactivate endogenous peroxidases with hydrogen peroxide.	Hydrogen peroxide
	Inactivate endogenous phosphatases with levamisole.	Levamisole
Endogenous biotin	Block endogenous biotin.	Incubate with streptavidin, followed by free biotin.
Ionic or hydrophobic interactions	Include detergent in buffers, optimize salt concentrations and pH.	Tween 20 and/or Triton X-100

Want more information?

Scan the code to access more information online!

www.jacksonimmuno.com