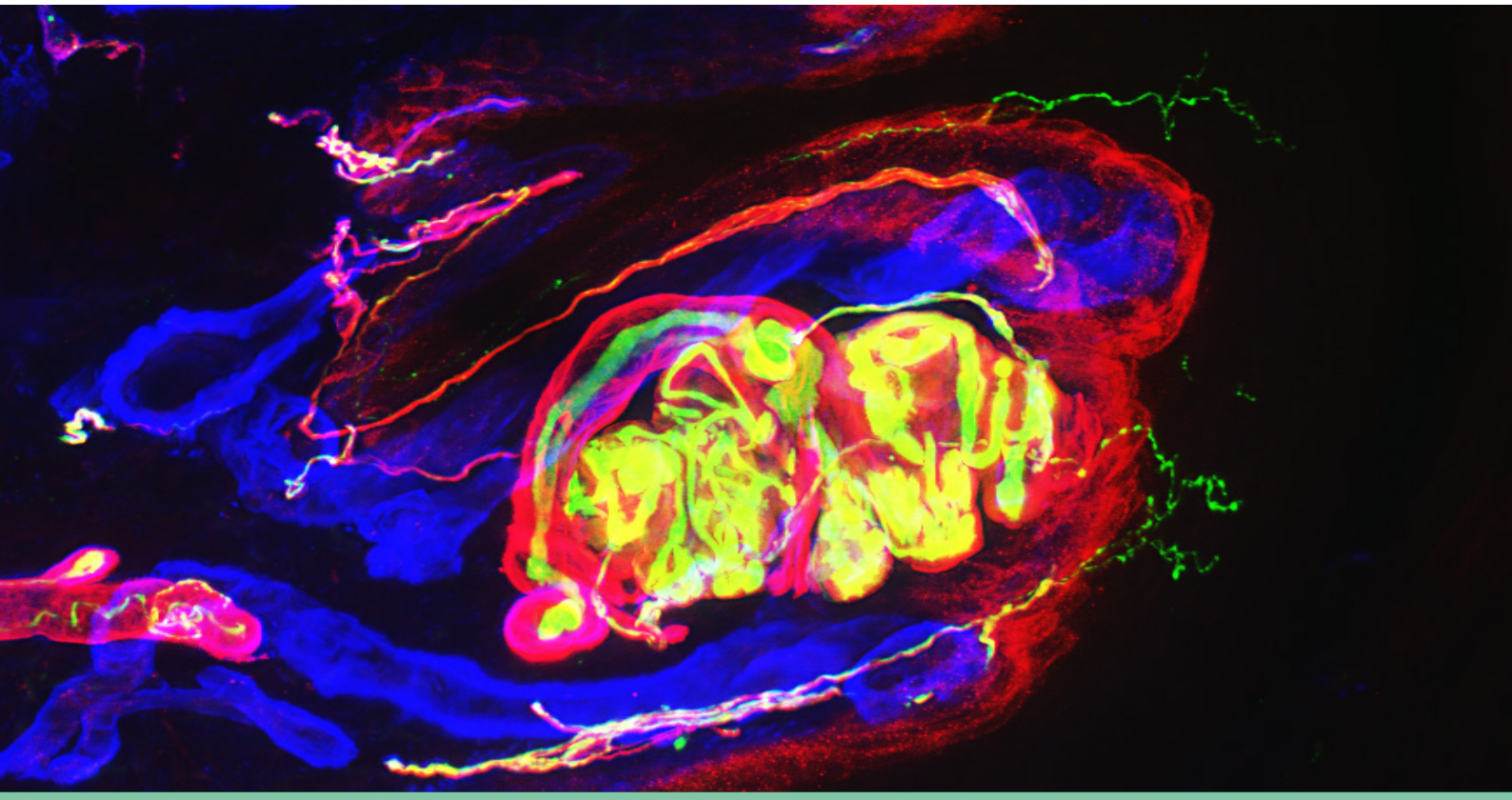
Jackson ImmunoResearch LABORATORIES, INC.

Specializing in

Secondary Antibodies and Conjugates



A guide to selecting control, diluent and blocking reagents

Optimize your experimental protocols with Jackson ImmunoResearch Secondary antibodies and Immunoreagents

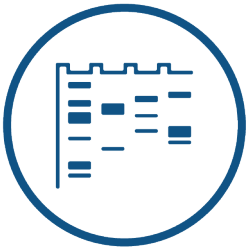
Optimize your experimental protocols with control, diluent and blocking reagents.

Experimental protocols using immunotechniques can often be improved through the optimal use of blocking reagents, diluents, and controls. Read more to learn about how to select appropriate diluents and blocking steps to abrogate unwanted background, and how experimental controls can help identify the source of the off-target signal.

When developing an immunotechnique it's important to consider how the results will be interpreted. Even the simplest assay can benefit from the addition of the correct blocking reagents and experimental controls. The inclusion of positive controls may be important if the images are to be used in publication. Depending on the technique employed, it may also be useful to add tissue and reagent controls. Diluent composition may also influence assay performance.

Here we detail a selection of common problems that can be solved with appropriate blocking, diluents or control reagents.

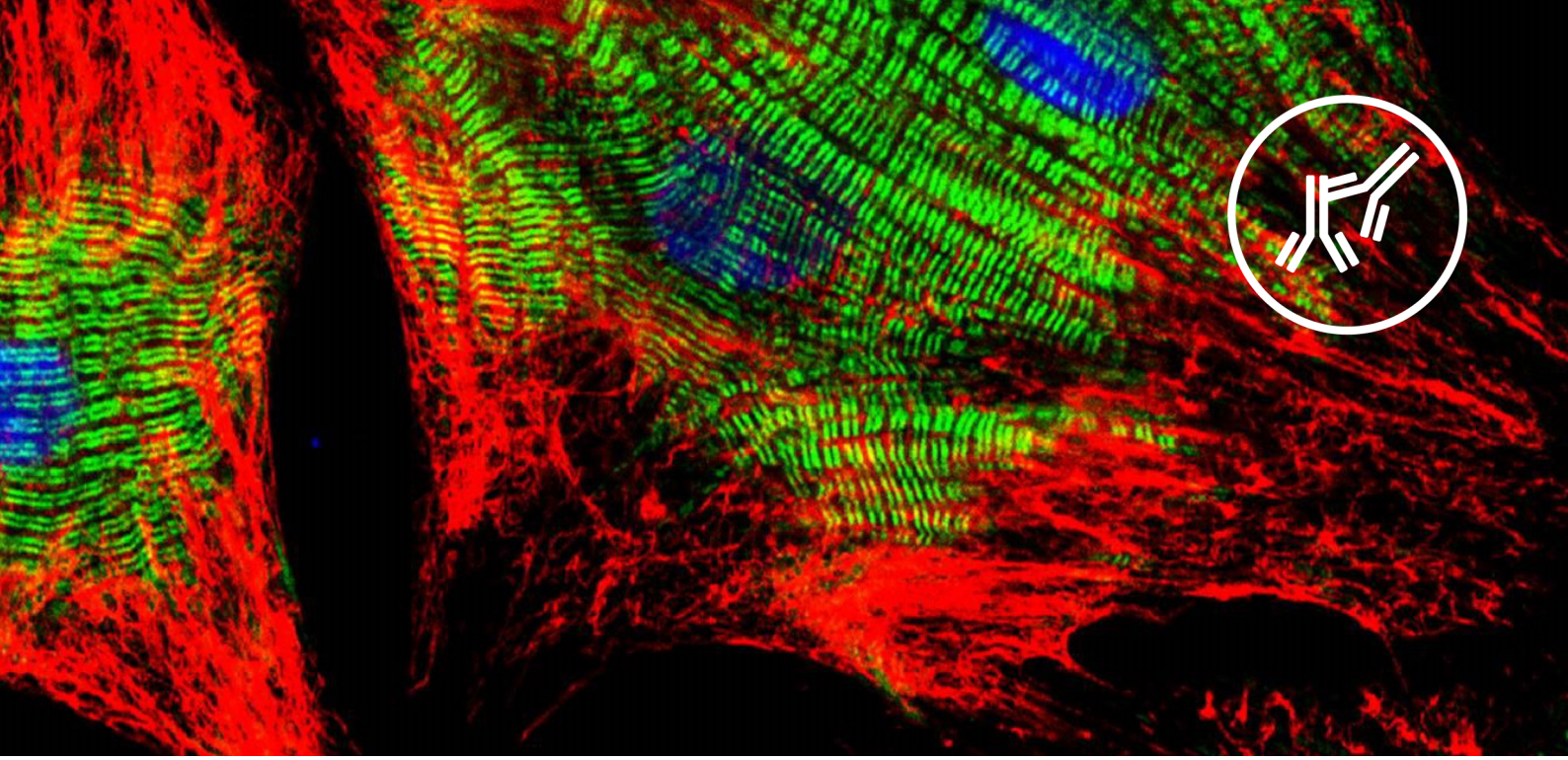
Western blotting



Problem	Solution	Indicated product
Background (non-specific signal obscuring bands of interest)	Use appropriate blocking reagent to block membrane prior to incubating with primary antibody.	Normal serum (5% v/v) from the host species of the labeled antibody, or BSA (IgG-free and protease-free).
	Avoid using milk or BSA if using primary antibodies derived from goat, horse or sheep. Bovine IgG may interact with the antibody due to homologous epitopes of closely related species.	5% v/v normal serum from the host species of the labeled antibody.
Detection of reduced immunoprecipitating (IP) antibody at 50 or 25 kDa	To avoid detecting IP antibody heavy chains at 50 kDa probe blot with conjugated anti-Light Chain specific antibody.	Anti-Light Chain antibodies (for more information see online or catalog).
	To avoid detecting IP antibody light chains at 25 kDa, probe blot with conjugated anti-IgG, Fc fragment after blocking with monovalent Fab fragment anti-Fc.	Anti-Fc antibody. See online or catalog for more information. Fab fragments.

For more information on troubleshooting your Western blot, see our troubleshooting guide online.

If you are experiencing problems with your Western blotting our technical team would be delighted to help you. You can email them at tech@jacksonimmuno.com.

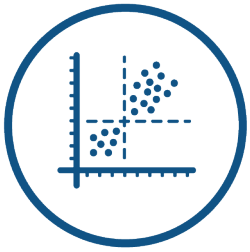


Immunohistochemistry, Immunocytochemistry, Immunofluorescence

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.
Background (homologous Ig recognition)	Block endogenous immunoglobulins.	Fab fragments
Multiple labeling of primary antibodies from same host species	Utilize Fab fragments in suggested protocols to accomplish multiple labeling.	Fab fragments
	Immunolabel primary antibody prior to incubation.	FabuLight™

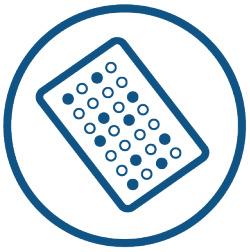
To confirm reporter enzyme activity, add a small sample of conjugated secondary directly to substrate and observe expected reaction.

Flow cytometry



Problem	Solution	Indicated product
Background from antibodies binding Fc receptors.	Block Fc receptors	Normal serum from the host of the labeled antibody. For more information see online.
	Use $F(ab')_2$ format secondary antibody to avoid entrapment by Fc receptors.	$F(ab')_2$ secondary antibodies, for more information see online.
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

ELISA



Problem	Solution	Indicated product
Background	Use appropriate blocking reagent to block wells prior to incubating with the primary antibody.	Normal serum (5% v/v) from the host species of the labeled antibody, or BSA (IgG-free and protease-free).
No signal	Use a positive control to demonstrate activity of the labeled secondary antibody coat with primary antibody isotype and detect directly with secondary antibody.	ChromPure purified proteins

Cautions:

When using a labeled secondary antibody for detection, never block with normal serum or IgG from the host species of the primary antibody. If immunoglobulins in normal serum bind to the specimen of interest, they will be recognized by the labeled secondary antibody, resulting in higher background.

Bovine serum albumin (BSA) and dry milk, both commonly used for blocking, may contain bovine IgG. With the exception of Bovine Anti-Goat IgG, many secondary antibodies such as Anti-Bovine, Anti-Goat, and Anti-Sheep will react strongly with bovine IgG. Therefore, use of BSA or dry milk for blocking or diluting these antibodies may significantly increase background and/or reduce antibody titer. For blocking, use normal serum (5% v/v) from the host species of the labeled secondary antibody.

Jackson ImmunoResearch Immunoreagents

Jackson ImmunoResearch offers a wide variety of immunoreagents designed to improve assay performance and ease of analysis.

Blocking reagents allow you to prevent background signal from endogenous proteins or non-specific interactions with substrates (e.g. ELISA plates, beads, tissue). Assay development and troubleshooting can be improved with the application of experimental controls.

ChromPure™ Purified Proteins from Normal Serums

ChromPure proteins are primarily used as experimental controls for either primary or secondary antibodies. They are available conjugated to a range of fluorescent dyes and reporter enzymes, allowing the isolation of signal derived from non-specific interactions. ChromPure proteins are derived from the serum of non-immunized animals and do not recognize any known antigens. They are prepared using a variety of chromatographic techniques to yield material with no contaminating molecules observed up to a concentration of 20 mg/ml, making them ideal for use as experimental controls for the most sensitive of assays. ChromPure purified proteins may also be used as blocking reagents for Western blotting, IHC and IF.

ChromPure purified proteins from normal serums are available in a variety of formats for many species , including whole immunoglobulin, F(ab ')$_2$ and Fab fragments. Human IgM , serum IgA and other proteins are also available. We offer a wide range of fluorescent and reporter molecule conjugates which are detailed in the table below. For a comprehensive list please see our catalog or online.

Format	Species	Non Fluorescent Molecules	Fluorophores
IgG (H+L)	Bovine	Unconjugated	Dylight 405
IgG F(ab ')$_2$ Fragment	Cat	Biotin-SP	Brilliant Violet 421™
IgG Fab Fragment	Chicken	HRP	Brilliant Violet 480™
IgG Fc fragment	Dog		Cy™2
IgM (myeloma)	Donkey		Alexa Fluor® 488
Serum Ig A	Goat		FITC
Albumin	Guinea Pig		Per-CP
Transferin	Hamster		R-PE
	Horse		Cy™3
	Human		Alexa Fluor® 594
	Mouse		Cy™5
	Rabbit		APC
	Rat		Alexa Fluor® 647
			Alexa Fluor® 680
			Alexa Fluor®790

IgG-Free, Protease-Free Bovine Serum Albumin

Bovine serum albumin (BSA) is used extensively as a carrier protein to dilute antibodies and as a general protein blocking agent in immunoassays and immunodetection protocols. If BSA is the desired diluent or blocking reagent for your assay it's important to know if the BSA is suitable for your assay.

Not all BSA products commercially available are comparable.

Most BSA products, including those marketed as having no detectable IgG, are contaminated with low levels of bovine IgG. The contaminating IgG shares many epitopes with goat, sheep and horse IgG, and can become a target for secondary antibodies directed against those species (an exception is bovine anti-goat IgG). This may occur with other antibodies that cross-react with bovine IgG as well. The interaction may result in loss of antibody activity, reduced antibody stability, and/or increased background. The background may derive from sticky soluble immune complexes or from non-specific binding from contaminating bovine IgG attracting cross-reacting labeled secondary antibodies.

Jackson ImmunoResearch Bovine Serum Albumin is IgG-free and protease-free. It does not contain contaminating IgG, which alleviates common immunoassay problems such as background and reduced signal associated with many commercial high purity preparations of BSA. IgG-free BSA is supplied as a pure protein, freeze-dried from deionized water in 10g, 50g and 250g pack sizes.

Description	Code Number	Fill Size
Bovine Serum Albumin (IgG-Free, Protease-Free)	001-000-161	10 g
	001-000-162	50 g
	001-000-173	250 g

Please see our catalog or website for more information.

Normal Serums

Normal serums are obtained from non-immunized animals, and consequently do not detect any specific antigen. Normal serum diluted to 5% (v/v) in PBS is strongly recommended as a blocking agent to reduce background from nonspecific, conserved-sequence, and/or Fc-receptor binding. Best results are obtained with diluted normal serum from the same host as the labeled antibody, used as a separate incubation step before addition of the primary antibody.

Secondary antibody host species	Suggest product	Suggested product codes	Vial size
Alpaca	Normal Alpaca serum	028-000-001	2ml
		028-000-121	10ml
Bovine	Normal Bovine serum	001-000-001	2ml
		001-000-121	10ml
Donkey	Normal Donkey serum	017-000-001	2ml
		017-000-121	10ml
Goat	Normal Goat serum	005-000-001	2ml
		005-000-121	10ml
Mouse	Normal Mouse serum	015-000-001	2ml
		015-000-120	5ml
Rabbit	Normal Rabbit serum	011-000-001	2ml
		011-000-120	5ml
Rat	Normal Rat serum	012-000-001	2ml
		012-000-120	10ml
Sheep	Normal sheep serum	013-000-001	2ml
		013-000-121	10ml

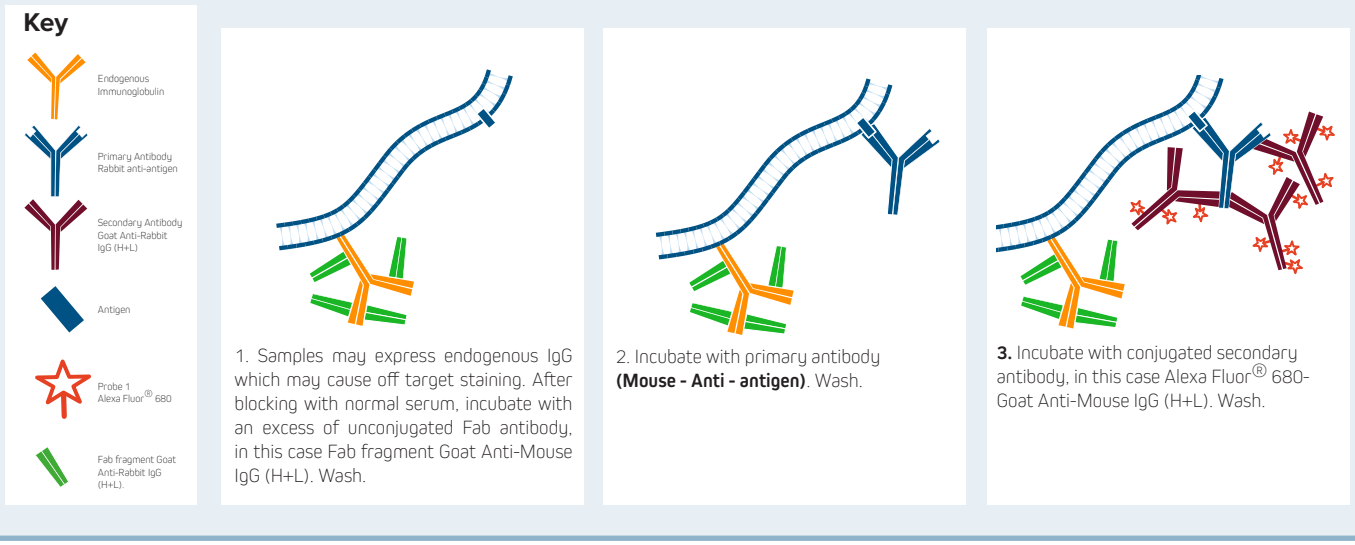
For our complete range of Normal Serums see our catalog or online.

Monovalent Fab Fragment Affinity Purified Antibodies

Fab fragments can enable the blocking of endogenous immunoglobulins to reduce background staining, and can be used for multiple labeling assays when primary antibodies are derived from the same host species.

Fab fragments can be used to block endogenous immunoglobulins to reduce background staining, and for double labeling primary antibodies from the same host species. The following example shows how fab fragments can be used to block endogenous immunoglobulins when using mouse primary antibodies on mouse tissue.

Example: Use unconjugated fab fragments to block endogenous immunoglobulins to avoid off target signal.



See online for more Fab fragment labeling protocols.

FabuLight™

FabuLight antibodies are Fab fragment secondary antibodies specific to the Fc region of IgG or IgM primary antibodies.

They are available conjugated with 9 different fluorophores and biotin, and enable labeling of primary antibodies prior to incubation with cells or tissue.

This provides a **time-saving alternative to sequential incubation** for flow cytometry and immunohistochemistry procedures, without compromising the active site of the primary antibody.

FabuLights may also be used for labeling cell surface immunoglobulins without cross-linking and activating B cells, and labeling Fc chimeras (fusion proteins). FabuLights are not provided cross-adsorbed against other species, so blocking steps may be required to avoid labeling endogenous immunoglobulins.

Learn more about FabuLight antibodies in our catalog or online.

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Picture credits.
Primary heart tissue cultures showing fibroblasts, stained for tubulin (red 712-165-153), cardiomyocytes stained for sarcomeric alpha-actinin (green 111-225-144), and red and blue (desmin positive 712-165-153) smooth muscle cells. Dr E Ehler, (2017) Kings College London, UK.

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