WHITEPAPER

Blocking Reagents:

The Building Blocks for Clean Staining

BIOCARE MEDICAL

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To obtain the most specific detection of antigens by immunohistochemistry (IHC), it is essential for all non-specific binding sites be blocked. Even an antibody with high specificity for its target can face challenges, as intermolecular forces can promote non-specific binding to other molecules. If these non-specific sites are left unblocked, the antibodies and detection system may bind them, leading to background staining and even prevention of true antigen-antibody binding.

As antibodies and detection systems used in IHC are mostly protein-based reagents, they are susceptible to non-specific protein binding. Non-specific antibody binding to endogenous Fc receptors or a combination of ionic and hydrophobic interactions can also lead to background staining. Protein blockers mitigate this erroneous binding activity by competing for the non-specific protein binding sites on the specimen. Blockers can utilize serum solutions, protein solutions, or proprietary protein-free compounds in order to do this. When applying serum blockers, serum from the source species of the secondary antibody is used. For example, goat serum would be utilized if the protocol had a goat anti-mouse secondary. In the case of protein-based blockers, the purified milk protein casein is popular. Biocare Medical offers serum and serum-free blockers for both manual and automated use.

While insufficient blocking leads to background staining, excessive blocking can interfere between antibody-antigen binding. For non-specific interactions to be reduced and antibody-antigen binding to not be limited, optimization is required to ensure the highest signal-to-noise ratio is achieved. A high ratio of signal to noise indicates a well optimized assay.

Biocare Medical's Endogenous Protein Blocking Reagents

Reagent Name	Catalog Number	Description
Background Punisher	BP974	• Universal casein block • Proprietary combination of proteins can be used with both human and animal tissue • Automated or manual staining
Background Sniper	BS966	• Universal casein block • Formulated with recombinant blocking proteins and is serum free. • Can be used with any detection system and on both human and animal tissues and for cell culture techniques.
Background Eraser	BE965	• PBS Solution of 10% goat serum and Tween 20 • Optimized to work with 4plus avidin-biotin detection system and when the secondary antibody is derived in goat
Background Terminator	BT967	• Goat serum with surfactant blocker • Use when excessive background exists • Designed for use with Biocare's MACH 2 detection, not suitable for two-step detections such as MACH 3, MACH 4, PromARK™ or intelliPATH™ detection.
V-Blocker	BRI4001	• Universal blocking reagent • One of the most effective blocking reagents for automated IHC systems • Can be used in the conventional manner (by applying before the primary antibody); however, using V-Blocker after the primary antibody and before detection has shown to be much more effective, especially when using double stain or multiplex applications.

To learn more about Biocare Medical's protein blocks, please call 800-799-9499 or visit our website:
https://biocare.net/products/ancillaries/blocking-reagent/