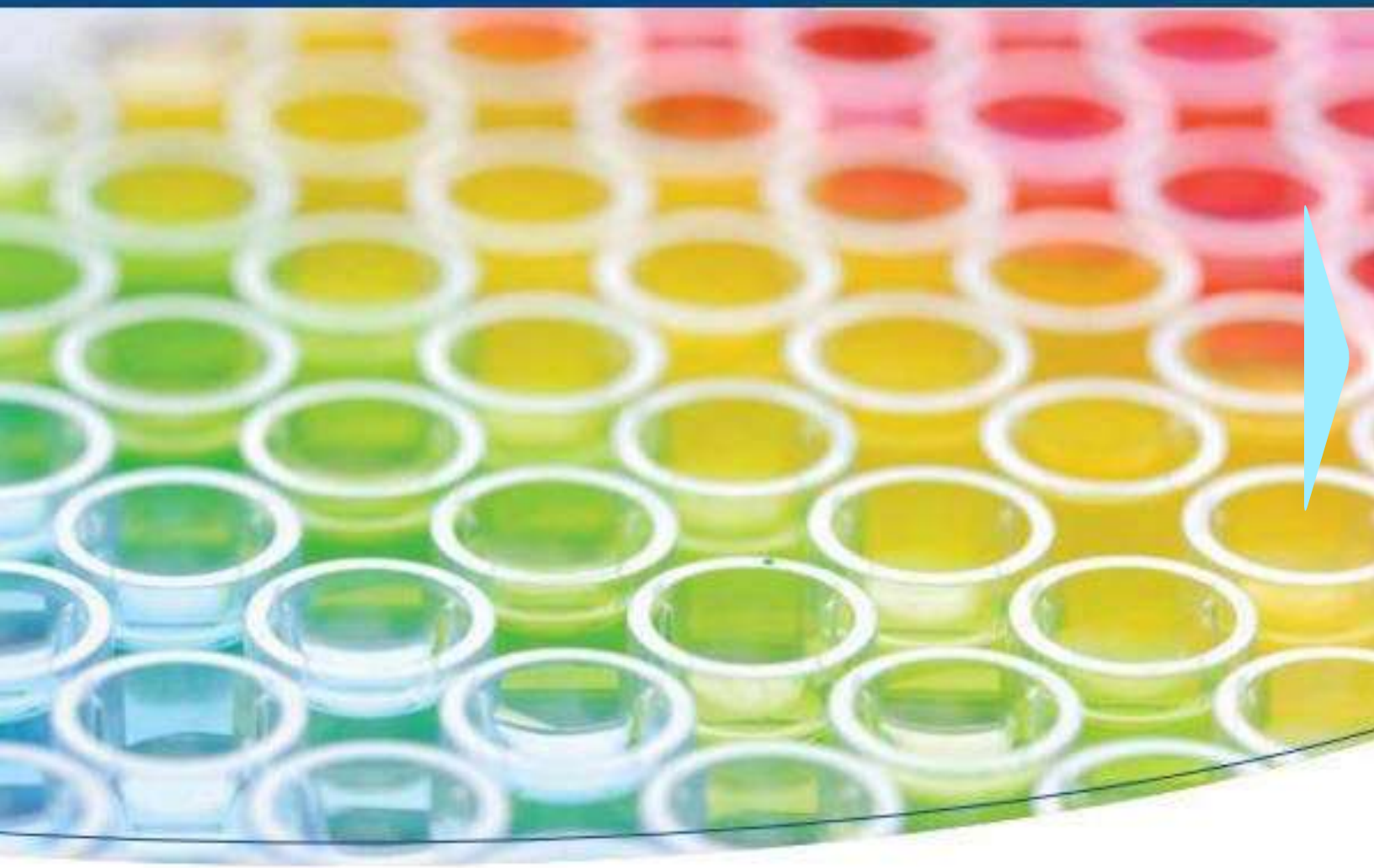
amsbio

Immunoassay Blocking Reagents

A Practical Guide



Blockers are used in ELISA and LF assays to reduce interference from proteins in patient samples that could produce false results and an incorrect diagnosis.

Choosing the best assay format depends on the intended application of the assay, the type of samples to be analyzed, the availability of reagents, and whether the assay is intended for a single analysis in one lab, or is intended to be used in many laboratories by various technicians. Generally, an immunoassay will fall into one of the following categories:

- **Sandwich Antigen Detection ELISA** - designed to measure the amount of target antigen/analyte
- **Antibody Capture ELISA** - used to screen for antibodies (e.g: IgG, IgM, IgA & IgE) to a specific target
- **Competitive ELISA** - detects antigen/analyte present in a sample and is commonly used when the antigen is small with only 1-2 epitopes.

Several parameters that are critical to assay performance are common among all of these formats: (1) the choice of solid phase (2) the choice of antibodies/antigen and (3) the choice of blocking agents. In order to produce an assay with high sensitivity and specificity, the most critical element is selecting antibodies or antigens that have a highly specific interaction with the target molecule. However, it is possible to improve assay sensitivity and specificity with the use of blocking agents. These blockers work by reducing non-specific binding to increase the signal-to-noise ratio.

Non-specific interactions in an assay can occur (1) between the solid phase and non-target proteins, which can absorb to the surface of the solid substrate and (2) between antibodies within the assay itself and endogenous antibodies present within a patient's sample. To prevent non-specific binding, blocking buffers are used after the solid-phase coating step to block any remaining open binding sites. Other blocking agents are used in the sample preparation to prevent interfering antibodies from binding to the assay antibody components.

Types of Immunoassay Solid Phases

Material	Binding Capacity	Type of interaction
Nitrocellulose	High	Hydrophobic, Hydrophilic
PVDF	High	Hydrophobic
Nylon	High	Hydrophobic
Plates and Tubes		
Polystrene	Low	Hydrophobic
Polyvinyl	Low	Hydrophobic
Derivatized microtiter plates	Low	Covalent, Hydrophobic, Hydrophilic
Beads		
Polystyrene		
Polystyrene	Moderate	Hydrophobic
Derivatized Polystyrene	High	Covalent, Hydrophobic, Hydrophilic
Microparticles	High	Covalent and Hydrophobic

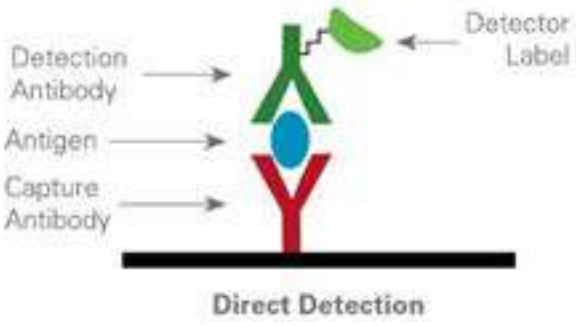
A solid surface which has a high binding capacity but does not cause damage to the native protein conformation of the immobilized antigen or antibody is ideal.

Key performance differences between monoclonal and polyclonal antibodies

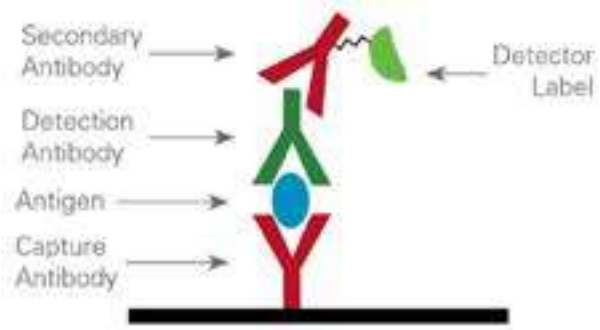
In general, a MAb is often chosen as the primary antibody to establish the highest level of specificity in an assay, and a PAb is chosen as the secondary antibody, to amplify the signal via multiple binding events. However, any combination can be used. All candidate antibodies must be tested together with the intended sample type in order to select the best performers.

Monoclonal antibodies (MAb)	Polyclonal antibodies (PAb)
• Generally produced in mice or recombinantly, these antibodies recognize a single epitope. • Since only one antibody molecule can bind to the antigen, the interaction is highly specific but can lack sensitivity.	• Produced in goats, sheep, chicken, rabbits and other animals. • Polyclonal sera is a heterogeneous composite of antibodies with unique specificities and the concentration of specific antibody (PAb) is typically 50-200mg/mL. • PAbs are able to recognize multiple epitopes on any one antigen which makes them less sensitive to antigen mutational changes. • PAbs are useful when the nature of the antigen is not well known. However, their quantity is limited by the lifespan of the animal.

Sandwich Antigen Detection Immunoassays



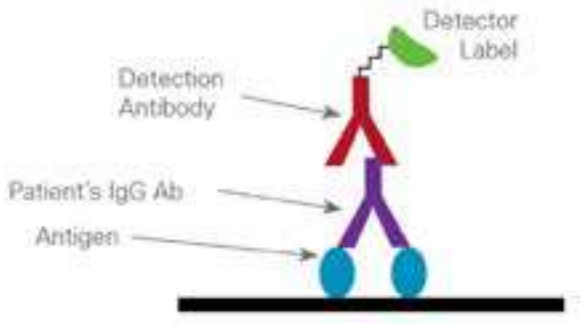
Direct Detection



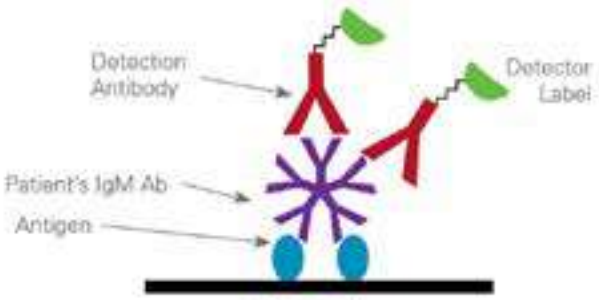
Indirect Detection

This is a sensitive and robust method which captures the target antigen between two antibodies (capture and detection antibody). The capture antibody is pre-bound to the solid phase and the antigen-containing sample is applied. A wash step is performed to remove unbound antigen and a detection antibody is added that binds directly to the antigen. The capture and detection antibodies must bind to non-overlapping epitopes on the antigen. Either monoclonal or affinity-purified polyclonal antibodies can be used as capture and detection, and the antigen can be measured with a conjugated detection antibody (direct detection) or a matched set of unlabeled detection and conjugated secondary antibodies (indirect detection).

Antibody Capture Assay (IgG, IgM & IgA)



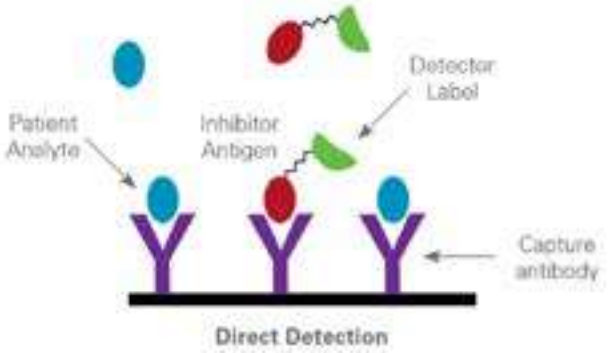
IgG Detection



IgM Detection

The antigen is immobilized on the solid phase by direct absorption and the antibody-containing sample is applied. Detection of the antibody can then be performed using an conjugated detection antibody (direct detection) or a matched set of unlabeled detection and conjugated secondary antibodies (indirect detection). Direct detection is shown.

Competitive Assay



Direct Detection

A competitive binding process between the patient's target analyte and add-in antigen (inhibitor antigen). Antibody specific for the target analyte is coated onto the solid phase. Patient sample and labeled inhibitor antigen are incubated with the pre-coated antibody and compete for biding sites. Unbound labelled antigen/analyte is removed by washing. The more analyte in the sample, the less the labelled inhibitor antigen will bind to the antibody. Therefore, the weaker the assay signal, the higher the concentration of analyte in the patient sample. This is a common method for small antigens that have only 1-2 epitopes

Blocking Assay Interference

Solid phase quantitative immunoassays such as ELISA, lateral flow and Western blot all involve the immobilization of antibodies to a surface via covalent interactions. Non-specific binding to this surface by other proteins or biomolecules can reduce an assay's specificity and sensitivity. Solid phase blocking agents are specifically designed to saturate these unoccupied binding sites to prevent non-specific binding, while enhancing assay sensitivity.

When developing a new immunoassay, the first step is to optimize the antigen or capture antibody coating conditions on the solid phase in order to maximize the amount of protein coated. After coating, any remaining unoccupied binding sites must be blocked in order to prevent non-specific binding of subsequent reactants. An ideal blocking agent is typically protein-based as it is able to block both hydrophobic and hydrophilic sites on the solid phase. In addition, it can serve as a stabilizing agent and prevent denaturation while proteins react at the surface of the solid phase. The concentration of the blocker and the amount of blocking time must be optimized for each assay. Using inadequate amounts of blocker will result in excessive background and a reduced signal-to-noise ratio. Using excessive concentrations of blocker may mask antibody-antigen interactions causing a reduction of the signal-to-noise ratio.

Ideal blocking agents have the following characteristics:

› Effectively block nonspecific binding of assay reactants to the surface of the well

› Do not disrupt the binding of assay components that have been adsorbed to the well

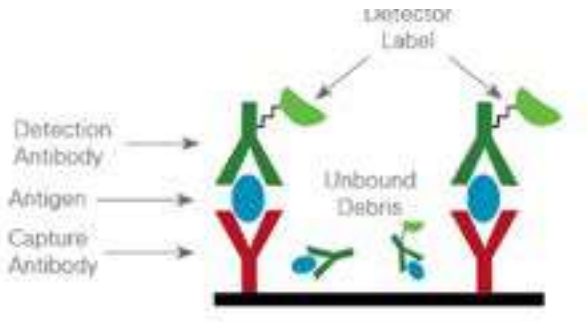
› Act as a stabilizer (prevent denaturation) of assay reactants on the solid phase

› Do not cross-react with other assay reactants

› Do not possess enzymatic activity that might contribute to signal generation of the substrate or degradation of the reactants

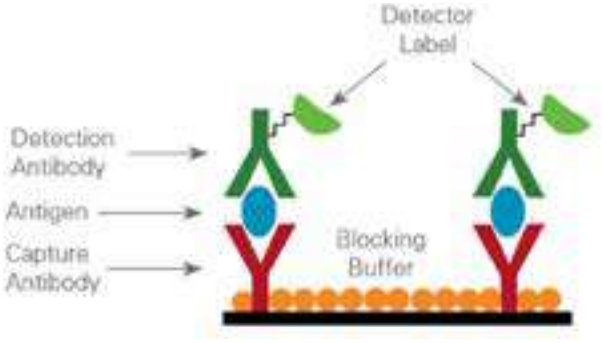
› Perform consistently across various lot

ELISA without blocking buffer



Unbound debris such as proteins, interfering molecules in the patient sample, and assay reactant bind to the solid phase and compete with the specific antigen-antibody reaction causing background noise and reduction in assay signal.

ELISA with blocking buffer



Blocking buffer is designed to bind to open sites on the solid phase preventing unbound debris from non-specifically binding.

Recommended Blockers

J82100B-500	**Blocking Buffer for ELISA, in PBS** A non-mammalian blocking reagent supplied in PBS (pH 7.4) for ELISA assay systems involving microplate or other solid phase material. Effectively blocks binding sites that remain on microplate or membrane following the initial coating steps. Provides an increased signal-to noise ratio. Use at 1x concentration or with further dilution.
J82300B-500	**Blocking Buffer for Lateral Flow, in PBS** A proprietary blocking reagent supplied in PBS (pH 7.4 ± 0.2) for lateral flow assay systems involving membrane or other solid phase material. Promotes a higher specific signal by minimizing non-specific binding and stabilizing bound proteins for maximum binding capacity. Uses non-mammalian protein blocking molecules. Use at 1x concentration or with further dilution.
J82200B-500	**Blocking Buffer for Western blot, in PBS** A non-mammalian blocking reagent supplied in PBS (pH 7.4) and optimized for use in sensitive Western blot assay systems. Formulated with non-mammalian proteins to prevent non specific interactions and improve assay specificity. Use at 1x concentration or with further dilution.
J16430D-125	**Coating Stabilizer and Blocking Buffer** A specially formulated reagent designed to improve the stability and function of antigens/proteins bound to a solid phase. Use as supplied for very unstable proteins or for less labile antigens/proteins. Dilute up to 1:1 in the assay's current blocker. Buffer has a pH 7.2 ± 0.2.
A64801B-50	**Bovine Serum Albumin (BSA)** The most common blocking agent and can be used for various applications including ELISA, Western blot, and IHC. BSA as a blocking reagent is particularly useful with casein-sensitive antibodies, such as phospho-specific antibodies. It is typically used at a concentration between 1% and 5% in PBS at pH 7.

General Blocking Protocol

1. After coating the solid-phase, add the blocking solution directly to the wells, beads, blotting membrane or nitrocellulose membrane.

2. Determine the best concentration of blocker for your assay. Blockers can be used at 1x concentration or diluted.

3. Determine the optimum incubation temperature and time for proper absorption of the blockers. Longer times and higher temperature increase the rate of blocking. Typical temperatures are 25°C - 30°C incubated for 30 minutes to 2 hours.

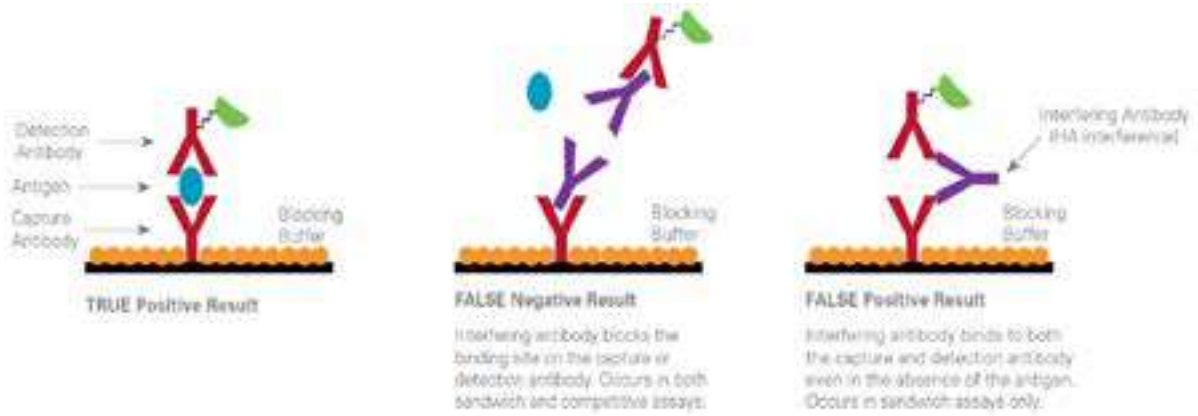
4. Proceed to wash steps.

NOTE: In addition to blocking, it is essential to perform thorough washes between each step. Washing steps are necessary to remove unbound reagents and decrease background noise. Insufficient washing will allow high background. A common technique is to use a diluted solution of the blocking buffer along with some added high-purity detergent.

Blocking Assay Interference

Immunoassay interference is a general term for substances that can change the outcome of an assay by causing a false positive or false negative test result. Examples of potentially interfering particles include endogenous antibodies or other binding proteins present in a patient sample, polyreactive antibodies or autoantibodies (heterophiles), and human anti-animal antibodies.

Interfering substances can have non-specific reactions that disrupt the reaction between the analyte and reagent antibodies in an immunoassay by either (1) out-competing the analyte of interest for binding to the assay antibodies (false negative) or (2) simultaneously binding to the assay capture and detection ant bodies in the absence of any analyte (false positive).



Assays that are inherently vulnerable to interference include double-sandwich antigen detection assays, competitive assays and IgM capture assays. Specific examples which have been reported in the literature are assays for ToRCH, CEA, CA-125, CK-MB, LH, FSH, prolactin, TSH, AFP, cardiac troponin I (cTnI) and hCG.

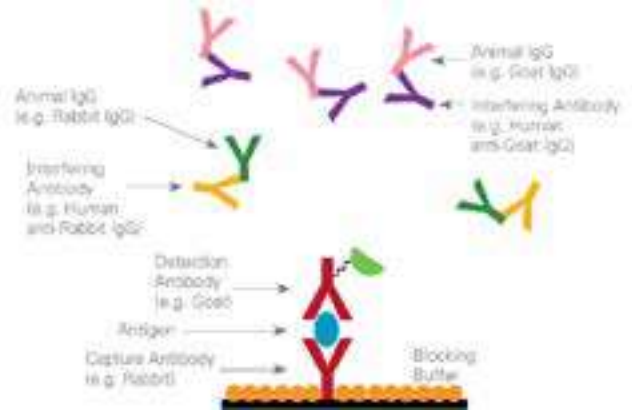
The most common type of interference in sandwich and competitive assays is heterophilic antibodies (HA) which are naturally occurring human antibodies with low affinities that can react with immunoglobulins from different species, including mouse, goat, rabbit, sheep and chicken. In diagnostic assays, HAs are able to bind to multiple and seemingly unrelated epitopes to disrupt the assay's specific antigen-antibody interaction. Human anti-mouse antibodies (HAMA) is one type of HA interference that specifically binds to mouse antibodies. Due to the high use of mouse monoclonal antibodies in commercial diagnostic immunoassays, HAMA interference is the most widely experienced type. Rheumatoid factor (RF), an autoantibody that reacts with the patient's own immunoglobulin (Ig), may also cross-react with animal Ig resulting in "RF interference", which is similar to HA/HAMA interference. Generally, isotype (Fc region)-specific interfering HA is more common than idiotype (Fab or binding site)-specific HA. IgM capture assays generally experience two types of interference. The first is from high levels of patient IgG antibodies that can compete with IgM for antigen binding sites on the solid phase. Since IgG antibodies are highly abundant, representing approximately 75% of serum antibodies in humans, they can outcompete IgM due to their sheer quantity. The second type of interference is caused by IgM RF which can produce false-positive signals by reacting with the Fc fragment on immunoglobulins. RF are found in 1 to 4% of the general population and in 75% of adult patients over 65 years of age.

A sandwich immunoassay uses two antibodies (either monoclonal or polyclonal) that bind to different sites on the antigen or analyte of interest. The capture antibody binding affinity for the antigen is usually the main determinant of immunoassay sensitivity. However, human anti-animal immunoglobulin antibodies (HA antibodies) can interfere with this interaction and reduce assay sensitivity and specificity.

ANIMAL IgG

Passive blocking reagents such as animal IgG work by preventing interfering antibodies from binding to the capture or detection antibodies by providing alternate binding sites. Animal IgG (e.g. Goat IgG) can only block one type of interference (e.g. human anti-goat antibodies) so typically more than one type must be used, depending on the host of both the capture and detection antibodies. Animal IgG must be added in excess concentration and the effectiveness depends on the affinity of interfering antibody for the animal IgG.

Recommended Concentration:
10x the concentration of the MAb/PAb being used in the assay (e.g. if 5mg/mL of Ab/conjugate, add 50mg/mL Animal IgG). Can be added to the sample or conjugate diluent but ideally should be in contact with the patient sample before incubation with the assay capture antibody.

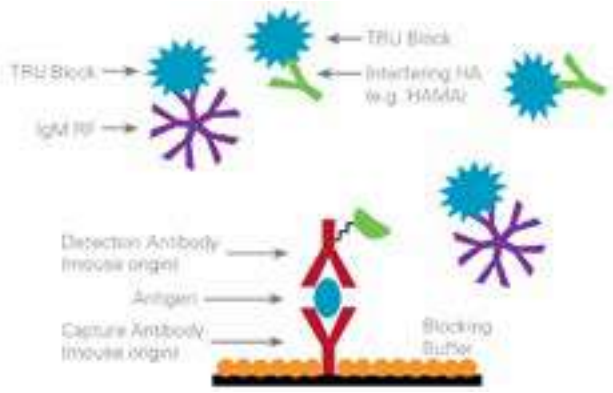


TRU BLOCK™ - HAMA & RF BLOCKER

In double mouse monoclonal assays, an additional blocker should be used to remove a specific type of HA interference called human anti-mouse antibodies (HAMA) and Rheumatoid Factor (RF). A HAMA blocker contains a specific binder directed against all types of heterophilic interference including HAMA and RF. Once bound to the interfering antibodies, TRU Block prevents further binding of HA to other assay components through steric hindrance. Active blockers can typically be used in lower concentrations than passive blocking reagents, which lessens the decrease in assay signal commonly associated with passive blockers.

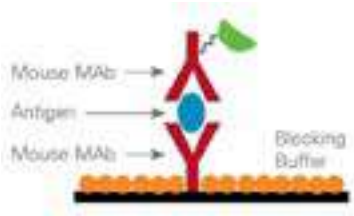
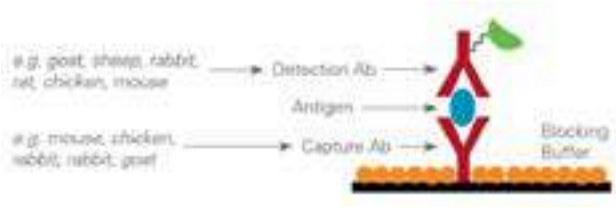
Recommended Concentration:
TRU Block should be included as part of the sample or conjugate diluent, at a recommended concentration range:

0.5mg/mL – 20mg/mL (diluted samples) or
5mg/mL – 20mg/mL (non-diluted samples)



Ideally TRU Block should be in contact with the patient sample before incubation with the assay capture antibody.

Choosing the Right Blocker

TYPE OF DOUBLE SANDWICH ASSAY		BLOCKER RECOMMENDED	
DOUBLE MOUSE MONOCLONAL	 	**TRU Block™**	**A66800H-0.1** (TRU Block 1) A66802H-0.1 (TRU Block 2) A66803H-0.1 (TRU Block 3)
		Mouse IgG	A66186M-0.02 (9-13mg/mL) A66185M-0.02 (50-55mg/mL) A66185M-LY-10 (Lyophilized)
MIXED SPECIES Capture and detection antibodies can be polyclonal or monoclonal		The species of IgG blocker used must be the same as the host of the capture and detection antibodies. More than one species of IgG is required when two different antibody species are used in a sandwich assay.	
Goat Polyclonal		**Goat IgG**	A66200H-1
Mouse Monoclonal		**Mouse IgG**	A66186M-0.02 (9-13mg/mL) A66185M-0.02 (50-55mg/mL) A66185M-LY-10 (Lyophilized)
Chicken Polyclonal		**Chicken IgY**	A01302C-50 (Lyophilized)
Rat Monoclonal		**Rat IgG**	A64391R-1
Rabbit Monoclonal or Polyclonal		**Rabbit IgG**	A66100H-1
Sheep Polyclonal		**Sheep IgG**	A66400S-0.05 (70-77mg/mL, Liquid)

Interference in IgM Capture Assays

IgM antibodies are the first type of antibodies produced by the immune system in response to an infection. Consequently, IgM detection assays have proven to be valuable diagnostic tools that assist in identifying early and recent infections. However, IgM antibodies only comprise 5% to 10% of all the antibodies in the body. In contrast, IgG antibodies are the most abundant immunoglobulin and comprise about 75% to 80%. In order to ensure an IgM assay is both sensitive and specific, it is necessary to reduce assay interfe ence, especially from the more plentiful IgG antibodies and other non-specific proteins, such as rheumatoid factors.

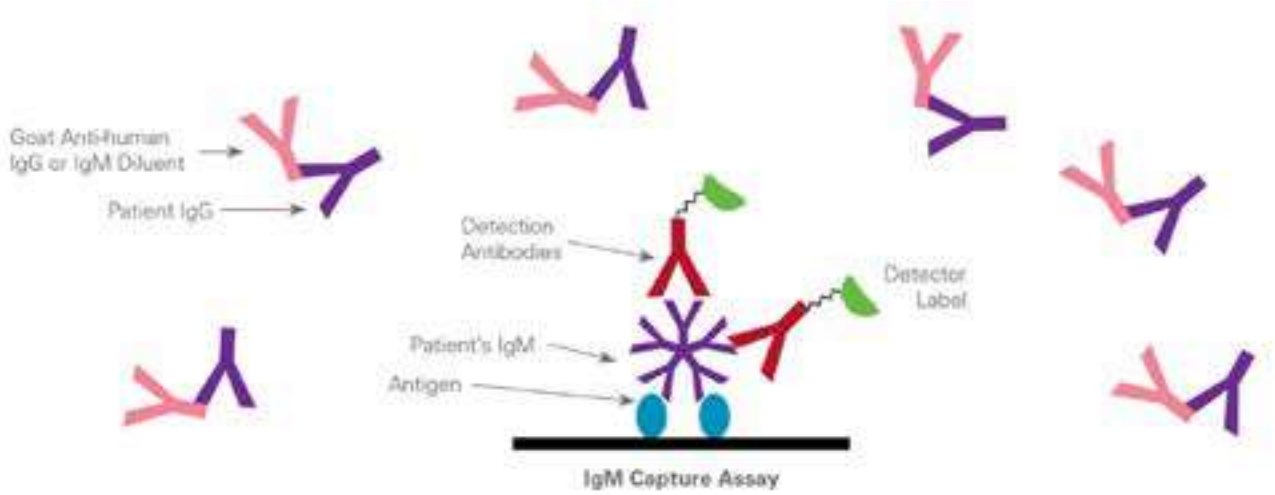
IgG ABSORBENT

IgG Absorbent is a purified goat anti-human IgG (GAH IgG) Fc fragment designed for the removal of human IgG and IgG/ rheumatoid factor (RF) complexes from serum prior to testing for specific IgM antibodies in ELISA or other immunoassays. Removal of IgG interference has been demonstrated to significantly increase the sensitivity of IgM detection in immunoassays. AMSBIO's IgG Absorbent is tested by immunoelectrophoresis to ensure specificity for IgG and no cross-reactivity to IgM or IgA.

IgM DILUENT

IgM Assay Diluent is intended for use in qualitative and quantitative assays that detect IgM antibodies. This diluent is formulated to reduce assay interference from a patient's IgG antibodies, rheumatoid factor, heterophilic antibodies, and other non-specific proteins that can affect the immunoassay results. Blocking proteins within the IgM Assay Diluent are in high excess and will bind to any open binding sites on the assay solid phase to prevent binding from interfering factors. When included as a part of a test kit, IgM Assay Diluent can increase the sensitivity of an IgM detection assay.

Recommended Reagents:	
L15406G-10	**Goat anti-human igG Fc (GaH igG)** Dilute prior to adding to patient sample. Recommend diluting 1:10 in PBS. Add in a ratio of 1:10 to patient sample and allow to incubate 5-30 minutes.
8120-25	**igM Diluent** In a separate tube, dilute the patient serum sample in the IgM Assay diluent at a 1:21 dilution or greater (mix well). The diluent must be standardized with the other assay components.I



ABBREVIATIONS

Ab	Antibody
Aff. Pur.	Affinity Purified
Block	Tested as a blocking agent
BSA	Bovine Serum Albumin
EIA, ELISA	Enzyme Immunoassay
Fc	Fragment crystallizable region of an antibody
H&L	Heavy and light chain in IgG
HA	Heterophillic antibodies
HAMA	Human anti-mouse antibodies
HRP	Horseradish peroxidase
IEP	Immunoelectrophoresis
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgY	Immunoglobulin Y
LF	Lateral flow
MAb	Monoclonal antibody
Monospecific	Monospecific when tested by immunoelectrophoresis
Neat	Whole, unpurified, undiluted antisera
Neph	Nephelometry
PAb	Polyclonal antibody
PBS	Phosphate buffered saline
Purified	Refer to the product COA regarding the extent of purification and/or process used
TIA	Turbidimetry
Tris	Tris(hydroxymethyl)aminomethane based-buffer

Animal Serums

Animal serum contains a diverse mixture of proteins which can absorb heterophile interfering agents in various assays. Animal serum can be used in place of or in addition to animal IgG. The species of animal serum must be the same as the antibody reagent in the assay.

Specificity	Type	Catalog #	Host / Source	Tested Apps	Format
Normal Bovine Serum, Sterile Filtred	Serum	N64007B-100	Bovine	Block	Neat
Bovine Serum, Sterile Filtred	Serum	N21010B-100	Bovine	Block	Neat
Fish Serum (cell culture grade)	Serum	N82800F-10	Fish	Block	Neat
Goat Serum, Sterile Filtred	Serum	N66001G-500	Goat	Block	Neat
Goat serum, sterile Filtered and Heat inactivated	Serum	N66111G-500	Goat	Block	Neat
Mouse serum, Filtered	Serum	N14010M-10	Mouse	Block	Neat
Mouse serum, Lipid-stripped	Serum	M0P20-500-1	Mouse	Block	Neat
Porcine serum, sterile Filtered	Serum	N64250P-1	Pig	Block	Neat
Rabbit serum	Serum	N66010R-100	Rabbit	Block	Neat
Rabbit serum, sterile Filtered and Heat inactivated	Serum	N01267R-500	Rabbit	Block	Neat
Rat serum, Filtered	Serum	N64110A-10	Rat	Block	Neat
Sheep serum, sterile Filtered	Serum	N21010S-100	Sheep	Block	Neat

Chicken IgY

Chicken IgY should be used in sandwich or competitive ELISAs or lateral flow assays that uses chicken antibody. Any human anti-chicken antibodies in the patient sample that could potentially interfere with the assay signal will be bind to the chicken IgY, preventing non-specific binding that could interfere with assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
Normal Bovine Serum, Sterile Filtred	A01302C-50	Chicken Serum	Block	Lyophilized
IgY, Chicken, 17-23 mg/mL	A01366C-10	Chicken Eggs	Block	Purified
IgY, Chicken, 10-50 mg/mL	A01460C-1	Chicken Serum	Block	Purified

Goat IgG

Goat IgG should be used in sandwich or competitive ELISAs or lateral flow assays that uses a goat antibody. Any human anti-goat antibodies in the patient sample that could potentially interfere with the assay signal can bind to the goat IgG, preventing non-specific binding that could interfere with the assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
igG, Goat - Differential precipitation, 8-12 mg/mL	A66200H-1	Goat Serum	Block	Purified

Mouse Immunoglobulins

Mouse IgG should be used in sandwich or competitive ELISAs or lateral flow assays that uses a mouse antibody. Any human anti-mouse antibodies (HAMA) present in the patient sample that could potentially interfere with the assay signal can bind to mouse IgG, preventing non-specific binding that could interfere with the assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
IgA, kappa, Mouse	A03090M	Mouse Myeloma	IEP	Aff.Pur.
IgG1, kappa, Mouse	A03090M-1	Mouse Myeloma	IEP	Purified
IgG2a, kappa, Mouse	A03117M-1	Mouse	IEP,ID	Purified
IgG, Mouse - Caprylic Acid Cut	A66187M-0.02	Mouse serum	Block	Purified
IgG, Mouse igG Protein a, 9-13 mg/mL	A66186M-0.02	Mouse serum	Block	Purified
IgG, Mouse - Protein a, 22-28 mg/mL	A66181M-0.02	Mouse serum	Block	Purified
IgG, Mouse - Protein a, Lyophilized	A66185M-LY-10	Mouse serum	Block	Lyophilized
IgG, Mouse - Protein a, 50-55 mg/mL	A66185M-LY-10	Mouse serum	Block	Purified
IgE, Mouse	A66185M-0.02	Mouse	IEP,ID	Aff.Pur.
IgE (Chimeric)	A03900M-0.5	Mouse	EIA	Supernatant
IgM, kappa, Mouse	A87180H-2	Mouse Myeloma	IEP,IP	Purified
IgM, lambda, Mouse	A03169M-1	Mouse Myeloma	IEP,IP	Aff.Pur.

Rabbit IgG

Rabbit IgG should be used in sandwich or competitive ELISAs or lateral flow assays that uses a rabbit antibody. Any human anti-rabbit antibodies in the patient sample that could potentially interfere with the assay signal can bind to rabbit IgG, preventing non-specific binding that could interfere with the assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
IgG, Rabbit, 8-12 mg/mL	A66100H-1	Rabbit serum	Block	Purified
IgG, Rabbit - Differential ppt	A66105R-1	Rabbit serum	Block	Purified

Rat IgG

Rat IgG should be used in sandwich or competitive ELISAs or lateral flow assays that uses a rat antibody. Any human anti-rat antibodies in the patient sample that could potentially interfere with the assay signal can bind to rat IgG, preventing non-specific binding that could interfere with the assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
IgG, Rat	A64391R-1	Rat serum	Block	Purified

Sheep IgG

Sheep IgG should be used in sandwich or competitive ELISAs or lateral flow assays that uses a sheep antibody. Any human anti-sheep antibodies in the patient sample that could potentially interfere with the assay signal can bind to sheep IgG, preventing non-specific binding that could interfere with the assay results.

Specificity	Catalog #	Host / Source	Tested Apps	Format
IgG sheep - caprylic acid/sodium sulfate, 45-55mg/mL	A66441S-20	Sheep Serum	Block	Purified
IgG sheep - Protein a, 5-15 mg/mL	A66551S-10	Sheep Serum	Block	Purified
IgG sheep, ≥70mg/mL	A66400S-0.05	Sheep Serum	Block	Purified

Solid Phase Blockers

Solid phase blocking agents are designed to saturate unoccupied binding sites on the solid phase to prevent non-specific binding. Typically blocking buffers are able to block both hydrophobic and hydrophilic sites on the solid phase. In addition, they can serve as stabilizing agents and prevent denaturation as proteins react at the surface of the solid phase.

Specificity	Catalog #	Host / Source	Tested Apps	Format
Blocking Buffer for eLisa, PBS based	J82100B-500	-	EIA, WB	-
Blocking Buffer for Lateral Flow assays, PBS based	J82300B-500	-	LF	-
Blocking Buffer for Western blot	J82200B-500	-	WB	-
BSA, Standard Powder	A64801B-50	-	EIA	Purified
Coating Stabilizer & Blocking Buffer	J16430D-125	-	EIA	Liquid
Conjugate stabilizer Diluent, HRP	J16200D-125	-	EIA	Liquid
Gamma Globulin, Bovine	A01245B-0.1	Bovine	IEP	Purified
Gamma Globulin, Bovine	A51300B-10	Bovine Serum	IEP	Purified

IgG Absorbents

IgG absorbents are designed for the removal of human IgG and Ig / rheumatoid factor (RF) complexes from serum and plasma prior to testing for specific IgM antibodies in ELISA or other immunoassays. Removal of IgG interference has been demonstrated to significantly increase the sensitivity of IgM detection.

Specificity	Type	Catalog #	Host / Source	Tested Apps	Format
igM Assay Diluent	-	8120-25	Goat	EIA	Purified
IgG (Fc)	PAb	L15406G-10	Goat	EIA,IEP,Neph,TIA	Monospecific
IgG (Fc)	PAb	G5G16-048-2	Goat	EIA	Aff.Pur.
IgG (Fc)	PAb	L62540G-10	Goat	IEP	Purified

TRU Block - HAMA & RF Blocker

In double mouse monoclonal sandwich assays, an active blocker against human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) should be used to prevent non-specific interference leading to false assay results. TRU Block is a proprietary active blocker that can significantly reduce assay interference and improve assay results. It can be used at a lower concentration than passive blocking reagents such as mouse IgG.

Specificity	Catalog #	Tested Apps	
TRU Block	A66800H-0.1	Block	Purified
TRU Block 2	A66802H-0.1	Block	Purified
TRU Block 3	A66803H-0.1	Block	Purified

HAMA & RF Testing Samples

HAMA (human anti-mouse antibodies) or rheumatoid factor positive patient samples that can be used to test the effectiveness of a blocking agent in any type of assay.

Specificity	Catalog #	Host / Source	Tested Apps	Format
Human anti Mouse antibody (HaMa) Plasma	A01431H-1	Human	EIA	Neat
Human anti Mouse antibody (HaMa) serum > 600 ng/mL	N01268H-1	Human	EIA	Serum
Rheumatoid Factor Plasma	A01270H-1	Human	EIA	Neat

Suggested Protocols

TRU Block™

Important recommendations for evaluating TRU Block against another HAMA Blocking Reagent:

1. Applicable to any sandwich immunoassay that use two mouse monoclonals. HAMA blocking reagents already present in the kit buffers must be removed prior to testing.
2. For optimum performance it is best to have the HAMA blocker in contact with the sample prior to the monoclonal antibodies. This is best accomplished by adding the HAMA blocker to a sample diluent. If your assay does not use a sample diluent, the HAMA blocker may be added to the conjugate diluent before performing a 1- step assay (simultaneous incubation of sample and conjugate in the test wells). In lateral flow assays, the HAMA blocker may be added to the conjugate pad, to a sample diluent or pretreatment buffer, or applied to the membrane as a blocking stripe located before the test stripe.
3. Suggested starting range for dilution series:
(1) For assays that use diluted samples – 20mg/mL to 0.5mg/mL
(2) For assays that do not use diluted samples – 200mg/mL to 5mg/ML.
4. Remove the current HAMA blocker from any diluents/buffers used in the assay before evaluation of TRU Block.
5. Include Positive Controls (Unblocked HAMA Samples) and Negative Controls (Buffer only - No HAMA Sample) in each assay.
6. Select only Human Serum or Plasma samples for evaluation that contain known interference by HAMA, HA and RF. Non-human (plant or animal) samples, feces, urine or other non-blood samples do not contain HAMA and are not appropriate for evaluation of HAMA blockers. When testing purchased samples, it is important to understand if the interference in your assay is caused by HAMA, HA, or RF in the sample. Assay interference may also be caused by non-specific binding or some other type of interference.

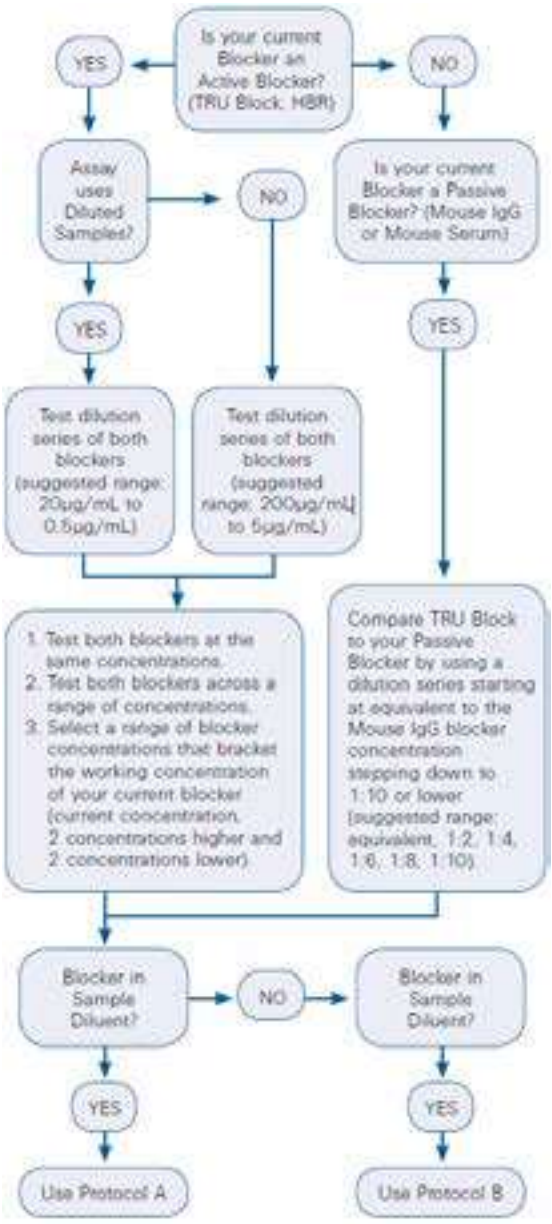
Protocol A: TRU Block in Sample Diluent

1. Prepare conjugate diluent buffer without blocker.
2. Dilute TRU Block in sample diluent buffer across a range of working concentrations (suggested starting range: 5mg/mL – 0.5ug/mL)
3. Dilute HAMA sample(s) with Sample Diluent containing blockers
4. Dispense diluted HAMA sample(s) in test wells
5. Incubate (according to the specific assay protocol)
6. Wash test wells
7. Dispense conjugate in test wells
8. Incubate (according to the specific assay protocol)
9. Wash test wells
10. Add substrate to test wells
11. Incubate (according to the specific assay protocol)
12. Add Stop Solution
13. Read OD Values
14. Calculate the percent HAMA signal blocked

Protocol B: TRU Block in Conjugate Diluent

1. Prepare conjugate diluent buffer without blocker
2. Dilute TRU Block in conjugate diluent buffer across a range of working concentrations (suggested starting range: 5mg/mL – 0.5ug/mL)
3. Dispense HAMA sample(s) in test wells. Add prepared conjugate diluent
buffer to buffer containing both conjugate diluent and TRU Block.
4. Incubate samples and conjugate/TRU Block in test wells simultaneously (according to the specific assay protocol)
5. Wash test wells
6. Add substrate to test wells
7. Incubate (according to the specific assay protocol)
8. Add Stop Solution
9. Read OD Values
10. Calculate the percent HAMA signal blocked

TRU BLOCK PROTOCOL SELECTION FLOW DIAGRAM



Comparing TRU BlockTM to your current blocker:

1. Perform a side-by-side evaluation of TRU Block to current blocker.
2. Follow protocol #1 or #2 using the same working concentrations of both TRU Block and your current HAMA blocker.
3. Calculate the percent HAMA signal blocked using the following equation:

$$\% \text{ HAMA Blocked} = 100 \times \frac{1 - (\text{Mean Blocked HAMA} - \text{Mean Negative Control})}{(\text{Mean Unblocked HAMA} - \text{Mean Negative Control})}$$

4. ANALYSIS: At each concentration, compare the percent HAMA blocking achieved by each blocker. The blocker that consistently achieves a higher percent HAMA blocking at a lower concentration is the most suitable for the assay.
NOTE: To quantify the percent blocking difference between blockers or blocker concentrations, the most sensitive assays will test the HAMA samples with unblocked HAMA activity in the linear range of the assay. For example, samples with very high unblocked HAMA/RF signals can be diluted to ensure the unblocked signal is within OD 1.000 - 2.000 (the linear range of the assay).

Mouse IgG

Recommended Usage:

1. For optimum performance, Mouse IgG should be in contact with the patient samples prior to contact with monoclonal antibodies in the assay. Anti-mouse antibodies in the patient sample will bind to purified Mouse IgG and will be blocked from binding to the monoclonal antibodies used in the assay.
2. Use Mouse IgG by placing it in the Sample Diluent, the Conjugate Diluent, or drying it down as a blocking stripe ahead of the test stripe in a Lateral flow assay.
3. Mouse IgG should be added at 10x the concentration of the monoclonal antibodies being used in the assay (example: if using 5ug/mL of MAb/conjugate, add 50ug/mL Mouse IgG). This makes the patient samples' anti-mouse antibodies 10x more likely to bind to the mouse IgG and not interfere with the assay MAbs.
4. Each assay format is different; the optimal working concentration and location of the blocker in the assay must be determined for each specific application.

Goat Anti-Human IgG (GAH IgG)

Recommended Usage:

1. Suitable for use in many different assay types including Nephelometric, Turbidimetric, ELISA, and Lateral Flow assays. For optimum performance, the patient sample should be treated with the GAH IgG prior to its contact with the antigen in the assay.
2. Suggested dilution s for initial testing (approximate removal of >90% IgG and recovery of >80% IgM):
• 10% GAH IgG in PBS
• 1:10 patient sample + diluted GAH IgG
3. Each assay format is different; therefore, the optimal working concentration and location of the blocker in the assay must be determined for each specific application.
4. NOTE: GAH IgG recognizes the Fc portion of the IgG molecule, therefore it will not recognize IgM, IgA, IgD, or IgE. It will form complexes with patient IgG or RF/IgG complexes and render them unavailable for further binding in the assay. Since IgG may be present in the patient sample at higher levels than IgM, the assay has to be formulated to not detect IgG.

IgM Assay Diluent

Usage notes for preparing patient samples in the IgM Assay Diluent:

1. IgM Assay Diluent should be stored at 2°C-8°C. Diluent must be warmed to room temperature prior to use.
2. Dilute the patient serum sample in the IgM Assay diluent at a 1:21 dilution or greater. The diluent must be standardized as a unit with the other assay components.
3. It is recommended that the dilutions are performed in a separate tube. Mix well after diluting.
NOTE: IgM Diluent contains Sodium Azide. Each lot is tested for efficacy using an IgM assay procedure.

Suggested Protocols *Continued*

HRP Conjugate Stabilizer Diluent

Usage notes for preparing HRP Conjugates in the HRP Conjugate Stabilizer:
1. HRP Conjugate Stabilizer is supplied ready to use – it can also be diluted up to 1:1 (final use dilution of stabilizer) in a saline solution (0.9% NaCl) to give greater product economy without compromising performance.
2. HEPES and MOPS buffers are highly compatible with the stabilizing solution, but only very small amounts of phosphate buffered solutions may be added.
3. Accelerated stability studies can be performed for several days at 50°C–55°C to insure efficacy of the stabilizing solution with your particular application.
(See package insert for complete instructions)

Blocking Buffer for ELISA

Blocking Buffer for Western Blot

Blocking Buffer for Lateral Flow

1. Add the blocking solution directly to the water wells, blotting membrane or nitrocellulose membrane depending on the assay type being used. Use at 1X concentration or with further dilution. Each laboratory should determine an optimum working dilution for use in its particular application.
2. Incubate at room temperature or 30°C for 30 minutes to 2 hours.
3. Continue with your process and reagents according to the assay protocol.

Coating Stabilizer and Blocking Buffer

Recommended protocol for stabilizing and blocking immobilized proteins:
1. Coat surface with protein/antigen.
2. Wash 1x to remove excess and weakly adsorbed proteins.
3. Before the protein is allowed to dry, coat the surface with the Coating Stabilizer and Blocking Buffer, completely covering the bound material.
4. Incubate at room temperature for 15 - 60 minutes.
5. Aspirate or drain the excess stabilizer from the surface (do not wash the surface).
6. Dry the protein (preferably under vacuum). Recommended drying times are as follows:
a. Two hours under vacuum (< 100 micron).
b. Overnight in a humidity controlled chamber that registers < 15% humidity .
7. Package the bound antigen/protein in a sealed airtight container with desiccant.
The product is now stabilized for long-term storage at 2–8°C.
(See package insert for complete instructions)

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