

Buffers & Saturating agents

Interchim provides all kinds of buffers and saturating agents for immunoassays. Here is a selection of ready-to-use buffers and remarkable reagents. See Chapter F (Biochemicals section) for other components (i.e. phosphate and other mineral buffers, Tris and other organic buffers, NaCl and other salts, Hepes and other good's buffers).

Technical tip – What is the best buffer and blocking agent ?

Buffers :

Phosphate Buffered Saline (PBS) and Tris Buffered Saline (TBS) are the most popular buffers for incubation and washing steps of immunoassays. But for Alkaline Phosphatase systems, phosphate based buffers are avoided and TBS is then preferred while NaCl is omitted for the final substrate incubation. Preservatives such as sodium azide are avoided for peroxidase systems, and citrate phosphate is used for substrate incubation. Tween20 is often added to wash and incubation steps. Now, each technique and each lab may have specific requirements or protocols: many options exists and should be tested for optimal results.

Blocking agents :

Bovine serum albumin (BSA) and **dry fat-free milk** are the most popular saturants, and work in many standard techniques with Alk.Phos., HRP or Fluorescence. However they may also contain low level of Igs which reacts with anti-IgG (i.e. bovine, goat, horse, sheep) antibodies. Therefore, they are not recommended to block or dilute primary antibody of these species as they may significantly increase background and/or reduce secondary antibody efficiency. Many other agents are used, most of time of mammalian (i.e. casein-based), and as such have similar possible undesired background or cross-reactions.

Gelatin is a cheap alternative usually very efficient with chromogenic detections, but is found sometimes not convenient to use (should be melted), having the drawback to mask certain antigens, and not suitable for ECL detections.

Seablock agent is then recommended for critical applications, showing no cross-reactivity with mammalian immunoreagents.

Additionally, each saturant can unfortunately hide more or less some antigens, and there is no rule to forecast this! Many factors intervene. Attention should be paid for optimal results when: changing I or II Ab specie, switching to avidin/biotin detector, using different samples types (blood, purified membrane...), changing labels (i.e. from HRP/4CN to HRP/ TMB, or to a fluorescent from a luminescent system).

To conclude, there is no ideal or universal saturant. When your standard agent does not meet expected results (background, insufficient sensitivity, unspecific bands in blot,...), you should test several agents of different types, and optimize concentration and buffer.

Guide lines for buffers composition	ELISA	Blot	μArray	IHC	FCM	HRP/AP	Biotin	Fluor	Chemil/HRP
Saturating buffer (post-coating): Choose an inert protein (at 0.5 to 10%) in saline buffer (i.e. 150mM NaCl) in conjunction with a surfactant (0.01 to 1%).	+	+	+	+	N/A	+	+	+	++
Incubation and washing buffer: PBS or TBS with 0.5% of Tween20 is usually sufficient. One tenth dilution with the saturating buffer may be useful especially for incubation buffer.	+	+	+	+	+	+	+	+	++
Guide lines for saturating components									
. BSA , a popular saturating agent except for chemiluminescence. must be avoided in systems containing anti-bovin, goat, horse or sheep II Abs.	+++	++	+	++	+++	+++	+++	+++	-/+
. casein / Milk , also a very popular saturating agent in many detection systems, especially for chemiluminescence. Should be avoided in biotin based assays or in systems containing anti-bovin, goat, horse or sheep II Abs.	++	+ / +++ (chem)	+	+	++	+++	-/+	+++	+++
. Gelatin , less largely used (time consuming). Gives excellent results in some detection systems (i.e. AP/BCIP blotting) but merely suits chemiluminescence. Do not suit glass supports. May mask epitopes.	++	++ (Enz)	- / ++	- / +	+	+ / +++	+ / +++	?	- / +
. Normal IgG , useful for blocking certain types of background (i.e. du to cell receptors, rheumatoid or polyreactive antibodies in samples, ...)	+	+ / ++ (cell type)	- / +	- / +++ (cell type)	- / +++ (cell type)	+++	+++	+++	+++
. Tween20 , an efficient saturant and buffer additive to prevent unspecific binding (c)	+++	+++	+++	+	-	+++	+++	+++	+++
. Other components , i.e. normal sera, fish sera (see seablock) (c)									
. Special, formulated saturants (c)	(a)	(a)	(a)	(a)					

(a) special saturating buffers, optimized by techniques, are proposed in corresponding sections (ELISA, WB, MicroArray, IHC).

(b) IgG (0.1-1%) or serum (1-10%) may be included to washing and incubation buffers. They are useful for detection systems with labeled anti IgG Abs on certain cell types, notably when IgG Fc binding sites (as Ig receptors in cells, cell extracts) generate a high background (IHC and IHF techniques). May increase background and affect double anti Igs labelings. Saturating IgGs and sera should be irrelevant to the capture and detecton ab species. For example, rabbit serum (UP379060) and rabbit IgG (UP378416) for anti mouse detections (no cross-reactivity).

(c) Ask InterBioTech for other ingredients, and formulations. For example other non-ionic surfactants (Tween80, TritonX100,...) have also been used, but may affect cells or even sensitive proteins and protein complexes. PolyethyleneGlycol is a versatile blocker available in a number of sizes, configurations and charges.

Go to Formulated Buffers and saturating agents

[Protein-based](#) (SeaBlock, BioBlock,...) | [Protein-free saturants](#) | [Antibody diluents](#)

Buffers and buffer components

[PBS & TBS](#) | [Saturating Buffers](#) | [Detergents](#) | [Albumins](#) | [Caseins](#) & milk products | [Gelatins](#) | [Serum & IgGs](#) | [Others](#)

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interbiotech@interchim.com



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tel. 04 70 03 88 55 - Fax 04 70 03 82 60

Formulated Buffers for Immunoassays

Protein-based saturating and dilution buffers

■ SeaBlock & AquaBlock, non mammalian saturating agents

- Non-mammalian nature prevents interactions with immunoreagents (i.e. mammalian antibodies)
- Lower background – No unspecific bands
- Excellent to saturate high binding surfaces, and Glutaraldehyde activated Amine polystyrene (when BSA, casein and other agents are good but not excellent or even poor blockers).

SeaBlock and Aquablock are prepared from fish serum. Although the immune system of fish is similar to other vertebrates, fish antibodies are unlike those of mammals and do not cross-react.

Seablock agent overcomes non-fat milk, BSA, Gelatin, FBS... in most immunoassays, due to high molecular diversity. It is excellent at room temperature and below (as in living fish) to stabilize refrigerated or frozen proteins during immobilization or drying. It suitare optimized for chromogenic and (notably with the optimized formulations) chemiluminescent systems; and for nitrocellulose lateral flow assays.

SeaBlock, standard

UP40301A, 500 ml AYP1X0, 500 ml

¶ Excels as a blocker in ELISA and other solid phase immuno-assays. [Technical Sheet](#) ^(H)

SeaBlock, serum free

UPAP1370 (in PBS), 500 ml UPAP1380 (in TBS), 500 ml

¶ Optimized as a blocker for lateral flow applications. [Technical sheet](#) ^(H)

AquaBlock

UPAM7281, 500ml

¶ Optimized as a blocker in WB with ECL detection. [Technical Sheet](#) ^(H)

Other available protein-based saturating agents follow:

SuperBlock Blocking Buffers

A protein-based popular blocking buffer for rapid blocking of Western blots and ELISAs –blot in 10min, ELISA plate in 2 min-

SuperBlock Blocking Buffer in PBS 279670, 1L

SuperBlock T20 PBS Blocking Buffer RJ2840, 1L

SuperBlock Blocking Buffer - Blotting in PBS 241540, 1L

This blocking buffer has been optimized for use with precipitating substrates and yields a high signal-to-noise ratio in most applications.

SuperBlock Blocking Buffer in TBS 668180, 1L

SuperBlock T20 TBS Blocking Buffer RJ2850, 1L

SuperBlock Blocking Buffer - Blotting in TBS 660720, 1L

This blocking buffer has been optimized for use with precipitating substrates and yields a high signal-to-noise ratio in most applications.

SuperBlock (TBS) Blocking Buffer Dry Blend L79770, 1L

StartingBlock (PBS) Blocking Buffer

A versatile, first-intention blocking reagent in every system. Protein-based.

StartingBlock (PBS) Blocking Buffer FN0470, 1L

A protein-based blocker formulation in phosphate buffered saline (pH 7.5) for use in Western blotting and ELISA applications.

StartingBlock T20 (PBS) Blocking Buffer FN0471, 1L

Formulation includes 0.05% Tween-20 Detergent.

StartingBlock (TBS) Blocking Buffer FN0480, 1L

A protein-based blocker formulation in Tris buffered saline (pH 7.5) for use in Western blotting and ELISA applications.

StartingBlock T20 (TBS) Blocking Buffer FN0482, 1L

Formulation includes 0.05% Tween-20 Detergent.

Bio-Block Saturating agent

An economic standard blocker for western blotting with chemiluminescence detection.

This blocking buffer contains 0.5 % hannersten casein available in either PBS or TBS, and is optimized for positively charged nylon or PVDF membranes in nucleic acid or protein blotting applications.

BioBlock membrane blocking agent (in PBS) N1366A, 1 L

BioBlock membrane blocking agent (in TBS) N1365A, 1 L

[Technical sheet](#)

Other special protein-based saturants:

Protein Blocker (animal serum free) FM2165, 100 ml FM2166, 500 ml

Effective for IHC, ELISA, WB – does not contain animal proteins, phosphate (good fo AP detection), nor Azide.¶

Other formulations are available on inquire.

SeaGrow¶

Blocking Solution (High Efficiency Blocker) #110050,

SmartBlock, Ready-To-Use Blocker Free of BSA #113125, 125 ml | 250ml | 500ml

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Other special saturants – by supports

Super G Protein Preservatives

Super G Blocking Buffer

LO8970-1051100, 100ml

Optimizes the use of nitrocellulose for protein assays using fluorescence detection. Significantly reduce in fluorescence background while maintaining the high protein-binding capacity of porous nitrocellulose. Purified, protein-free solution has high binding efficiency for rapid and comprehensive clogging of non-specific protein. See section Micro-Arrays

Super G Plus Protein Preservative

105102, 100ml

Delivers highly efficient blocking of non-specific protein binding on polymer films, with additional components optimized for long-term protein preservation. Designed for MicroArrays, this formulation improves assay performance for various proteins stored for extended periods after immobilization on polymer films. [FS](#). See section MicroArrays.

Protein-free saturating agents

■ Protein-free Blocking Buffers

Eliminate or minimize cross-reactivity associated with protein-based blocking buffers. The need to match species with the secondary antibody is eliminated due to the lack of normal serum in these products. 1X ready-to-use formulations.

RapidBlock™ Solution, 10X

DZ7330, 15ml

DZ7331, 100ml

Specially designed for Blotting applications

Protein-Free (TBS) Blocking Buffer

RJ2860-37570, 1L

Proprietary formulation in Tris-buffered saline at pH 7.4 with Kathon Antimicrobial Agent

Protein-Free T20 (TBS) Blocking Buffer

RJ2870-37571, 1L

Proprietary formulation in Tris-buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent

Protein-Free (PBS) Blocking Buffer

RJ2880-37572, 1L

Proprietary formulation in phosphate buffered saline at pH 7.4 with Kathon Antimicrobial Agent

Protein-Free T20 (PBS) Blocking Buffer

RJ2890-37573, 1L

Proprietary formulation in phosphate buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent

Super G Protein Preservatives

Super G Blocking Buffer

LO8970-1051100, 100ml

Optimizes the use of nitrocellulose for protein assays using fluorescence detection. Significantly reduce in fluorescence background while maintaining the high protein-binding capacity of porous nitrocellulose. Purified, protein-free solution has high binding efficiency for rapid and comprehensive clogging of non-specific protein. See section Micro-Arrays

See also • [SeaBlock](#) serum free #UPAP1370
• [Components](#): TBS + Tween20 components

▪ [Super G](#) Blocking Buffer #LO8970

Antibody diluents, Preservatives

■ Antibody diluent Solutions

Universal antibody dilution buffer is ready to use for dilution of antibodies in all immunoassays (Immunofluorescence, IHC, ELISA, and WB). This buffer does not contain any mammalian proteins, phosphate, sodium azide or mercury preservative and can be used for dilution of all antibodies, including peroxidase, and antibodies to phosphoproteins. Not suitable for dilution of antibodies to S100 proteins. This buffer contains green food color. This buffer is also available with BSA (Ig free) as a stabilizer.

Universal Antibody Dilution Buffer, ready to use

DU4670, 100 ml

DU4671, 250 ml

DU4672, 500 ml

Universal Immuno Buffer 10X

DU4660, 100 ml

DU4661, 1L

Antibody Dilution Buffer (with BSA, Immunoglobulin free)

DU4680, 100 ml

DU4681, 250 ml

DU4682, 500 ml

Search online other formulations or [ask](#) your specifications. Ex.:

Universal Casein Diluent/Blocker #770627, 250 ml

■ LifeXtend™ HRP conjugate stabilizer/diluant

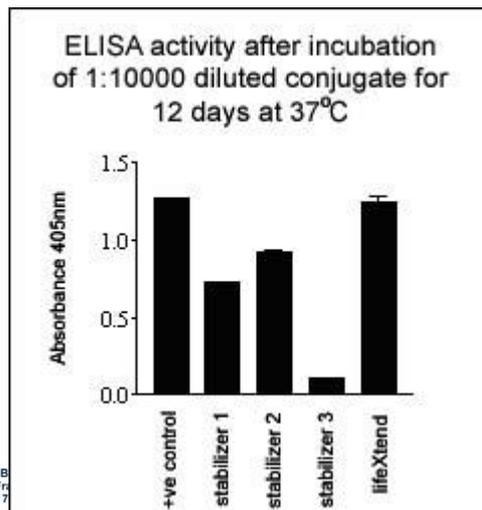
LifeXtend™ conjugate stabilizer/diluent is a proprietary multi-component reagent system that stabilizes antibody-HRP conjugates, as pre-diluted, ready-to-use reagents, during use and stored both at 4°C and at ambient temperature. This eliminates waste and improving consistency from experiment to experiment.

LifeXtend™ HRP conjugate stabilizer/diluant

YQ7070, 50ml

LifeXtend™ AP conjugate stabilizer/diluant

YQ7080, 50ml



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213 Avenue J.F. Kennedy - B
03103 Montluçon Cedex - Fr
Tel. 04 70 03 88 55 - Fax 04 7

Sample additives / diluants

HAMA blocker

This reagent blocks anti-mouse antibodies (wide range of epitopes) in patient samples, and so reduce the frequency of false positive and false negative results in immunoassays.

HAMA blocker

O25570, 20 mg

*contains purified Mouse IgG validated for anti IgG mouse/human interferences

*tested for all primary IgG isotypes, no contaminating species (bv, hu, rb, rt), nor proteinA, and proteases

RF Blocker

RF (Rheumatoid factor) blocker

B81ZK0

The RF (Rheumatoid factor) blocker contains a blend of anti-RF antibodies to block RF factors in blood samples once added before analysis. It prevent false positive results and give more biorelevant/accurate real positive results.

+

Search [more RF products](#)⁰, including serum/plasma/abs controls, diluent, ELISA kits..

Buffers and saturating components for IA – general use

Following are standard buffers used for Immunoassays (IA) -may be useful also other applications-.

■ Standard Buffers for IA (PBS, TBS)

PBS (Phosphate Buffered Saline) and TBS (Tris Buffered Saline) are surely the mostly used buffers for immunoassays.

See also [Biochemical catalog](#) for buffers components and additives (Azide, ...) as powders.

PBS powder pack (for 10L)	UP68723A, 1 pack	Technical Sheet
PBS liquid , 20X solution	N1376A, 500ml N1376B, 1 L	*
PBS tablets (1 Tab.makes 100-200-500-1000ml of 1X solution)	30715A, 100 Tabs@100ml	Technical Sheet
PBS with Tween® , pH 7.5	N13810, 500 ml	
TBS powder pack (for 20L)	UP74004A, 1 pack	
TBS liquid , 20X solution	N14580, 4 L	
TBS tablets (1 Tab.=100 ml of 1X solution)	GS3660, 100 Tabs	

•Other buffers are available [online](#) () or on inquire, i.e. 10X concentrate PBS (1P7120), D-PBS pH7.4 sterile solutions([GS3574](#))

See ou [Biochemical catalog](#) for individual buffering compound (i.e.

Milk, non fat, powder #768701

Potassium Phosphate Dibasic Anhydrous #687963 | Sodium Bicarbonate ACS Grade #70059A | Sodium Carbonate Anhydrous #141321 | Sodium Chloride #14139E | Sodium Dihydrogenophosphate #021050(Anhydrous) and 834861(Monohydrate) | Tris Base, UP0316570 | Tween® 20 #UP158743 and UP158740(oxidant free)

Coating buffers and components

•Standard coating buffers (pH 9.6, for proteins)

Coating buffer (for ELISA)	VD6700, 100 ml , 500ml
Coating buffer, 10X concentrate	J0719A, 50 ml

•Optimized and special coating buffers

Antibodies and antigens coated to polystyrene plates are more or less denatured by the passive coating procedure. In some cases they are even rendered non-functional and non-recognizable, notably in case of plate drying (ex: (strept)avidin in sensitive upon drying and loss its binding capacity – see precoated and stabilized sav-plates in the ELISA catalog, e.g. #UPL76161). For these applications, you might try:

UltraCoat ELISA Plate Coating Buffers help stabilize immobilized proteins to preserve three dimensional structure and functionality. They are designed for optimal coating of antibodies and antigens to polystyrene plates.

ULTRACOAT ELISA PLATE COATING BUFFER, 10X #AJ2570-C100, 100ml-500ml

Contains a physiological based solution of monobasic and dibasic phosphates. TS(L)

ULTRACOAT ELISA PLATE COATING BUFFER, 5X #XDK410-C1214, 30ml-100ml, 500ml, 1L, 10L

Proprietary composition. TS (L)

COATING BUFFER , PH 7,4 (10X) #120125, 125 ml-500ml-1L

•Other formulations are available [online](#) () or on inquire.

See ou [Biochemical catalog](#) for individual buffering compound (i.e. Tris #, Phosphate dibasi #, carbonate sodium #,

■ Ready to use Standard Blocking Buffers

Blocking solutions are high quality powdered prepackaged blends for use during immunodetection in various techniques, including ELISA and Blotting. They save your time (already weighed out in order each pouch will make one liter of solution) and are high proteomics grade for optimal results.

TBS with Non-Fat Powdered Milk 3%	GS4160, 5 pk (42 g/1 L)
TBS with BSA 1%	GS4170, 5 pk (22 g/1 L)
PBS with Non-Fat Powdered Milk 3%	GS4180, 5 pk (39.8 g/1 L)
PBS with BSA 1%	GS4190, 5 pk (19.8 g/1 L)

■ Detergents (Tween 20,...)

Several detergents are used in immunoassays, for their own saturating properties (i.e. Tween®20 may be sufficient to block microplates in ELISAs), but generally rather in conjunction with or secondly to a protein saturating agent. They are added to incubation and washing buffers (about 0.05%) to prevent unspecific binding of probes. Tween®20 is the most popular detergent for this purpose. Tween®80 has been useful for IgM applications. CHAPS, is a gentle detergent that may be useful in critical systems (weak immunologic affinities).

The oxidant free quality improves the stability of enzymatic conjugates during storage and incubations, and also favors more consistent inter-analysis results.

Tween® 20, pure **15874A, 1 L**
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Non-fat Milk 768701, 500 g
Non-fat Milk, proteomics grade GS4110, 10X10 g
Dry Milk Powder 76870A-22012, 4x25g

Also available:

Clear Milk Blocking Buffer (10X) 37587

a pre-formulated milk solution, clarified and stabilized for blocking excess nonspecific binding sites, reducing background in western blotting applications and diluting antibodies when used with nitrocellulose and PVDF membranes. It provides lower background, enhanced sensitivity, extended shelf life, and reproducible results compared to standard dry milk.

See also formulated buffer Bio-Block #N1365A

■ Gelatin

An excellent saturating agent for blots with chromogens. May however mask antigens.

Gelatin N13360, 100 g N13361, 500 g
CAS [9000-70-8]; Bloom number : 240-270 ; pH(28°C) : 4.5-5.5 ; Water (KF) : <12%; Viscosity : 35-45mpa

Fish Gelatin Blocking Agent 10X CN018A-22010, 100ml

Fish Gelatin Blocking Agent is a solution of cold water fish skin gelatin that can be added to blocking buffers to minimize non-specific antibody binding in immunodetection procedures such as Western Blotting and immunostaining. Unlike BSA or milk, fish gelatin does not contain IgG or serum proteins that could cross-react with mammalian antibodies.

See also [SeaBlock](#) saturating agents.

■ Serum and IgG based saturating agents

Normal -irrelevant- Sera and IgGs

These reagents are prepared from blood of healthy animals. They are typically used as controls or as additives in various immuno-techniques, notably to reduce non-specific detections in IHC and IF techniques.

Applications :

- Saturation of tubes, vials, gels, columns, or filters to prevent non-specific adsorption of diluted molecules
- Saturation of IgG binding sites to prevent non-specific adsorption of immuno-reagents in FCM, IF and IHC, ELISA.
- Saturation of IgG specific-binding sites for antibody-reagents:
Ig Fc receptors present on cells or in cell extracts, IgG-cross-reactive antibodies (i.e. Rheumatoid factors)
- Stabilization of reagents
- Protein controls and standards for assays or analysis (i.e. electrophoresis)
- Dilution of blood samples for dosage at constant protein concentration
- Equilibrium studies (free and bound fractions of a drug in serum)
- Co-precipitation: normal IgGs are added to precipitate a diluted monoclonal

Species	Normal Serum	Normal purified IgG
Bovine	UP89243C, 2 ml	
	UP89243A, 10 ml	
	UP89243B, 100 ml	UP757700, 10 mg
Cat	989140, 5 ml	869310, 10 mg
Chicken	UP37908A, 10 ml	773320, 5 mg
Dog	784110, 2 ml	
	784111, 5 ml	M08940, 10 mg
Donkey	UP77719A, 10 ml	866570, 10 mg
Goat	UP379031, 2 ml	
	UP379030, 10 ml	UP767090, 10 mg
GuineaPig U	P37916A, 10 ml	M09850, 10 mg
Hamster (Syrian)	UP28432A, 2 ml	826540, 10 mg
Horse	UP24741A, 10 ml	
	UP24741B, 100 ml	
	UP24741C, 500 ml	766730, 10 mg
Human	UP697906, 10 ml	UP408603, 10 mg
Mouse	UP379120, 2 ml	
	UP379121, 10 ml	UP386670, 5 mg
	UP37906B, 5 ml	UP378416, 10 mg
Rabbit	UP37911A, 2 ml	
Rat	UP37911B, 10 ml	UP443086, 5 mg
	UP697927, 10 ml	UP797560, 10 mg
Sheep	UP379021, 10 ml	
Swine	UP379022, 100 ml	UP062701, 10 mg

- **Normal serum :**
The serum is collected after blood coagulation. After addition of 0.09% of sodium azide as preservative, it is filtered using a 0.45µm filter.

- **Normal pure IgG :**
Immunoglobulin from class G (IgG) are highly purified from the serum of non-immunized animals. Uptima's pure IgG are provided in solution at 5mg/ml in PBS buffer with 0.09% sodium azide and filtered using a 0.45µm filter.

See also HAMA blocker #O25580 (avoids false positive and false negative results in immunoassays)

■ Other saturating agents and Buffers components

Prionex

BSA alternative, strongly reduces unspecific binding of protein to plastic microplate walls.

Prionex®, 10% sterile solution

901770, 100 ml

Go to previous and next sections of the PH catalog:

ImmunoDetection > [General Immuno-reagents^{\[PH\]}](#) > [Buffers and saturating agents^{\[PH\]}](#) (actual section)
 ImmunoDetection > [General Immuno-reagents^{\[PH\]}](#) > [Enzymatic Substrates^{\[PH\]}](#)
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