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 (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 recommanded to block or dilute primary antibody of these species as the 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 certains antigens, and not suitable for ECL detections. **Seablock** agent is then recommended for critical applications, showing no cross-reactivity with mammalian immunoreagents.

Additionnaly, each saturant can unfortunately hide more or less some antigens, and there is not rule to forcast 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, unsufficient 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 0.05 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 saturants									
. 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)	-/++	-/+	+	+/+++	+/+++	?	-/+
. Tween20, a very efficient saturant and buffer additive to prevent unspecific binding. May affect cells.(c)	+++	+++	+++	+	-	+++	+++	+++	+++
. Normal IgG and Sera. (b)	+	+/++ (cells)	-/+	-/+++ (cell type)	-/+++ (cell type)	+++	+++	+++	+++
. 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 certains 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 ab species (i.e different from I Ab specie). For example, rabbit serum (UP379060) and rabbit IgG (UP378416) for anti mouse detections (no cross-reactivity).

(c) other non-ionic surfactants (Tween80, TritonX100,...) have been used. PolyethyleneGlycol is a versatile blocker available in a number of sizes, configurations and charges

■ Standard Buffers for Immunoassays

PBS (Phosphate Buffered Saline) and TBS (Tris Buffered Saline) are surely the mostly used buffers for immunoassays.
See also Biochemical section for powders and additives (Azide, ...)

PBS powder pack (for 10L)	**UP68723A, 1 pack**
PBS liquid, 20X solution	**N13761, 1 L**
PBS tablets (1 Tab.makes 100 ml of 1X solution)	**307150, 100 Tabs**
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**

■ Ready to use Standard Blocking Buffers for Immunoassays

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**		
Tween® 20, 20% solution, oxidant free *	**UP158740, 5x10 ml**	**UP158741, 10x10 ml**	
Tween® 80, 20% solution, oxidant free *	**UP158780, 5 x 10 ml**	**UP158781, 10 x 10 ml**	
CHAPS	**UP333514, 5 g**	**UP333515 25 g**	**UP333516, 100 g**
CHAPS, Proteomics grade	**33351K, 5g**	**33351L, 10g**	**33351M, 50g**
TritonX100, 20% solution, oxidant free *	**UP521121, 5x10 ml**	**UP521122, 5x10 ml**	
n-Octyl-b-ThioGlucoside	**UP602080, 1g**	**UP602081, 5g**	

*Highly pure and packaged in sealed ampuls under argon to increase the accuracy of immunoassays.

See also BioClean detergent for labware cleaning.

■ Albumins

Bovine Serum Albumin (BSA) is a popular saturating agent used to block unspecific binding on microplates, blotting membranes and cell sections. We recommend our standard BSA as a first intention choice for most immunoblocking applications.
NB : please note that BSA does not suit correctly chemiluminescent detection and is not recommended for use with anti-goat or sheep secondary antibodies. (see Technical tip)

BSA standard grade for immunoassays:

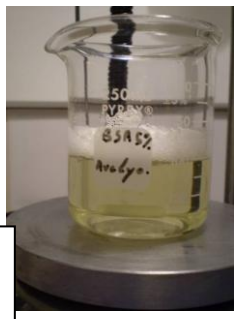
BSA powder **UPQ84170, 100 g** **UPQ84171, 500 g** **UPQ84172, 1 kg**
Our standard grade and economic BSA, ubiquitous for most biotechnologies, including immuno-saturations.

BSA 30% solution **UP900100, 50ml** **UP900101, 500ml**
More convenient than powders (no dissolution concerns, no aggregates), and cheaper than ready-to-use blockers
Using this solution, forget the hassle of weighting and dissolving BSA powder (no agregates!). Save time and money!

Technical tip – BSA solution is clearly the better solution!

Forget this
Fastidious weighting
Difficult handling / long dissolution

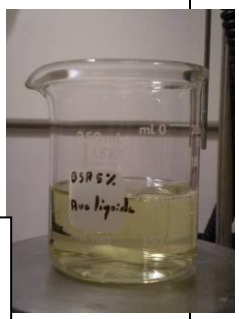
Detrimental foam
> biomolecules oxidation
Indesirables agregates
> artifacts on blots, background



5-15 min

and adopt Uptima BSA solution !
Pipette the BSA solution directly in your buffer
Dissolution is almost instantaneous

It's ready and clear !



<1 min

Other special grade BSA for demanding or specific applications:

BSA, IgGs and Proteases free **WU1640, 10g** **WU1641, 100grams**
For avoiding cross-specific reactions using anti IgG secondary antobodies. See also alternative blockers such as SeaBlock reagent.

Polymerised BSA, 30% solution (Immuno-Hemato grade) **BJ1450, 50ml**
See also our Prionex solution as a BSA alternative to reduce unspecific binding of protein to plastic microplate walls (see "Other saturating agents" paragraph below)

Find our BSA for cell culture, proteomics or genomics analysis in each related chapter.

Technical tip - **Caution for use of BSA and dry milk** with anti-goat or anti-sheep secondary antibodies.
BSA and dry milk may contain small amounts of bovine IgG which are very closely related to goat and sheep IgG. Anti-goat or sheep secondary antibodies will cross-react with bovine IgG and will significantly increase background. Therefore, the use of BSA or dry milk is not recommended to block or dilute neither these secondary antibodies nor goat and sheep primary antibodies.

See our BSA IgGs free reagent (opposite) and our SeaBlock, non mammalian blocking agent (below) and our Western Quick block kits (in our blotting chapter).

Ovalbumin:

Ovalbumin, from chicken egg white MW: 65KD; Ultrapure (>98%) llyophilized powder (M)	**R5851A, 1g**	**R5851B, 5g**

■ Casein (milk) based saturating agents

Fat-free Milk

Milk is an efficient and cheap saturating agent in immunodetection systems.
NB : please note milk should be avoided for streptavidin/biotin based separations or assays and is not recommended for use with anti-goat and sheep secondary antibodies. (see Technical tip)

Non-fat Milk	**768701, 500 g**
Non-fat Milk, proteomics grade	**GS4110, 10X10 g**

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)	**N13660, 1 L**
BioBlock membrane blocking agent (in TBS)	**N13650, 1 L**

■ 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 adsorbtion of diluted molecules
- Saturation of IgG binding sites to prevent non-specific adsorbtion 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
Contact your local distributor	InterBioTech, powered by	

- **Normal serum :**
The serum is collected aftr 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.

interbiotech@interchim.com
interchim 213 Avenue J.F. Kennedy - BP 1140 03103 Montluçon Cedex - France Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

Human	UP697906, 10 ml	UP408603, 10 mg
Mouse	UP379120, 2 ml	
	UP379121, 10 ml	UP386670, 5 mg
Rabbit	UP37906B, 5 ml	UP378416, 10 mg
Rat	UP37911A, 2 ml	
	UP37911B, 10 ml	UP443086, 5 mg
Sheep	UP697927, 10 ml	UP797560, 10 mg
Swine	UP379021, 10 ml	
	UP379022, 100 ml	UP062701, 10 mg

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

■ SeaBlock & AquaBlock, non mammalian saturating agents

- Non-mammalian nature prevents interactions with immunoreagents (i.e. mammalian antibodies)
- Lower background – No unspecifc 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 agent overcomes non-fat milk, BSA, Gelatin, FBS... in most immunoassays. It Suits chromogenic and chemiluminescent systems; and also available in special formulations for nitrocellulose lateral flow assays.

SeaBlock, standard **UP40301A, 500 ml**
excels as a blocker in ELISA
AquaBlock **UPAM7281, 500ml**
Excels as a blocker in WB
SeaBlock, serum free in PBS **UPAP1370 (in PBS), 500 ml** **UPAP1380 (in TBS), 500 ml**
excels as a blocker for lateral flow applications

■ 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.

Protein Block (animal serum free) **FM2165, 100 ml** **FM2166, 500 ml**
Effective for IHC, ELISA, WB
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

See also TBS + Tween20 components

■ Other saturating agents and Buffers components

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

Prionex

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

Prionex®, 10% sterile solution **901770, 100 ml**

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.

■ Antibody diluents

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 availble 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**

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**