

Immunologicals - Accessory reagents

Buffers & Saturating Agents

Technical tip

Buffers

Phosphate Buffered Saline (PBS) and Tris Buffered Saline (TBS) are popular buffers for most incubation and washing steps, but each technique and each lab may have specific requirements or protocols. For example in Alkaline Phosphatase systems, phosphate based buffers are avoided and TBS is preferred but 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 staining. Tween20 is often added to wash and incubation steps. Biological (hepes) buffers are helpful in specific applications.

Standard blocking agents

Saturating or blocking agents are used after the antigen coating step to prevent immunological binding on coating sites, and eventually in incubations and washing buffers. There is no good universal saturating agent. One agent that shows excellent results in a definite system may be mediocre (higher background, masked epitopes, ...) in another system. 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).

Interchim provides a large range of accessory reagents, from general use biochemical (buffers) to systems dedicated to specific techniques (i.e. microplates, blotters, LED).

Buffers & Saturating agents

Immunodetections require aqueous solutions, which are compatible with Ab/Ag binding, and enzymatic activity or fluorescence emission. Several biochemicals are used, starting with buffering agents (pH control), salts (physiologic ionic molarity), and saturating agents (blocking of non-specific binding sites).

Below are presented the popular PBS and TBS buffers including standard saturating components, then important saturating agents and additives for preparing your own buffers. Please search more Biochemicals in section "Biochemicals". See also detection kits by techniques, for ELISA, Blotting, IHC, that may include their own buffers or saturating agents.

Antibody diluent

Antibody diluent Solution is prepared for immunohistochemistry. Antibodies can be diluted directly in this solution and can be stored for up to 18 months when refrigerated (Higher dilutions might result in shorter shelf life).

This product contains 1% Bovine Serum Albumin in phosphate buffer saline, pH 7.4 and stabilizer.

Description	Cat.#	Qty
Antibody Diluent (Ready to Use)	HH6690	25 ml

Selection guide

The table below gives some guidelines to choose your saturating agent, and presents some classical ones. However in critical experiments, and for optimal results, a comparison of different blocking agents in your own conditions may be required. The most important criteria is usually to get the better signal-to-noise ratio.

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.	+	+	+	+	+	+	+	+	+
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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.	++	+ /+++	+	+	++	+++	-/+	+++	+++
• 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.		++	++	- /++	- /+	+	+ /+++	+ /+++	- /+
• Tween20, a very efficient saturant and buffer additive to prevent unspecific binding. May affect cells.(c)	+++	+++	+++	+	-	+++	+++	+++	+++
• Normal IgG and Sera. (b)	+	+ /++	- /+	- /+++	- /+++	+++	+++	+++	+++

Miscellaneous (c)(d) (a)

(a) special saturating buffers are usually provided with MicroArrays slides (see section 'MicroArray') - (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 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 (d) PolyethyleneGlycol is a versatile blocker available in a number of sizes, configurations and charges

Blocking Buffer Blends

Blocking Agents are high quality powdered prepackaged blends for use during immunodetection in various techniques, including ELISA and Blotting. These reagents include: TBS + Non-Fat Powdered Milk, TBS + BSA, PBS + Non-Fat Powdered Milk, PBS + BSA, and TBS + Tween® 20. All are proteomics grade, and have already been weighed out in order each pouch of Blocking Agents will make one liter of solution.

Description	Cat.#	Qty
TBS with Non-Fat Powdered Milk	GS4160	5 pk (42 g/1 L)
TBS with BSA	GS4170	5 pk (22 g/1 L)
TBS with Tween*	GS4200	5 pk (12.5 g/1 L)
PBS with Non-Fat Powdered Milk	GS4180	5 pk (39.8 g/1 L)
PBS with BSA	GS4190	5 pk (19.8 g/1 L)
PBS with Tween*	GS4250	5 pk (10.4 g/1 L)

Albumins

Bovine Serum Albumin (BSA) is a very popular saturant in many immunoassays. It however does not suit correctly to chemiluminescent detection. Products are available in powder, and in a convenient 30% solution

We recommend our standard BSA as a first intention choice for most immunosaturation applications. It is available in powder and in ready to use solution.

However a full range of other grades of BSA and other albumins are also available.

Description	Cat.#	Qty
BSA powder, standard grade	UPQ84170	100 g
	UPQ84171	500 g
	UPQ84170	1 kg

Our standard grade and economic BSA, ubiquitous for most biotechnologies, including immuno-saturations.

Description	Cat.#	Qty
BSA 30% solution	UP900130	50 ml
	UP900131	500 ml

More convenient than powders (no dissolution concerns, no aggregates), and cheaper than ready-to-use blockers

Casein (milk) based saturating agents

Milk is an efficient saturating agent in immunodetections systems, but it should be avoided for streptavidin/biotin based separations or assays. Bovine serum albumin (BSA) and dry milk may also contain IgG which reacts with anti-bovine IgG, anti-goat IgG, anti-horse IgG, and anti-sheep IgG antibodies. Therefore, use of BSA and/or dry milk to block or dilute these antibodies and/or your primary antibody may significantly increase background and/or reduce secondary antibody titer.

Description	Cat.#	Qty
Non-fat Milk	768701	500 g

Bio-Block Saturating agent

An economic standard blocker for western blotting with chemiluminescence detection. This blocking buffer is optimized for positively charged nylon or PVDF membranes in nucleic acid or protein blotting applications. Available in either PBS or TBS. (contains 0.5 % hannersten casein)

Description	Cat.#	Qty
BioBlock membrane blocking agent, in PBS	N13660	1 L
BioBlock membrane blocking agent, in TBS	N13650	1 L

Gelatin

Gelatin is used at 0.1-0.5% to saturate microplates and blotting membranes.

CAS [9000-70-8]
Bloom number : 240-270
pH(28°C) : 4.5-5.5
Water (KF) : <12%
Viscosity : 35-45mpa

Description	Cat.#	Qty
Gelatin	N13360	100 g
	N13361	500 g

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Buffers & Saturating Agents

Detergents (Tween 20,...)

Several detergents are used in immunoassays, with their own saturating properties (i.e. Tween®20 may be sufficient to block microplates in ELISAs), but generally they are rather used in conjunction with or secondly to a protein saturating agent. They are also frequently added to incubation and washing buffers (about 0.05%) to prevent unspecific binding of probes. Tween®20 is the most popular detergent for these purposes. 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.

Description in section "Biochemicals".

Description	Cat.#	Qty
Tween® 20, pure	15874A	1 L
Tween® 20, 20% solution, oxidant free	UP158740	5 x 10 ml
Tween® 80, 20% solution, oxidant free	UP158780	5 x 10 ml
CHAPS	UP333514	5 g
TritonX100, 20% solution, oxidant free	UP521121	5 x 10 ml

Serum and IgG based saturating agents

Sera and purified IgGs are useful in detection systems using labeled secondary Abs when undesired IgG bindings occurs generating high background (i.e. in IHC and IHF techniques) or non-specific detections due to :

- ◆ Ig Fc receptors present on cells or in cell extracts
- ◆ IgG-cross-reactive antibodies (i.e. Rheumatoid factors)

SeaBlock saturating agents

A non mammalian reagent containing saturating agent, in particular for chemiluminescent detections.

- ◆ Overcomes non-fat milk, BSA, Gelatin, FBS... in many immunoassays
- ◆ Suits immuno-enzymatic detection techniques , especially ELISA and Blotting
- ◆ Suits chromogenic and chemiluminescent systems
- ◆ Non-mammalian nature prevents interactions with immunoreagents (i.e.mammalian antibodies)
- ◆ Lower background
- ◆ Excellent to saturate high binding surfaces, and Glutaraldehyde activated Amine polystyrene (when BSA is respectively a good but not excellent or a poor blocker).
- ◆ Also available in special formulations (serum free in PBS or TBS buffer) for nitrocellulose lateral flow assays

Description	Cat.#	Qty
SeaBlock (standard, excels as a blocker in ELISA)	UP40301A	500 ml
SeaBlock (standard, excels as a blocker in WB)	UPAM7281	500 ml
SeaBlock, serum free in PBS (for lateral flow assays)	UPAP1370	500 ml
SeaBlock, serum free in TBS (for lateral flow assays)	UPAP1380	500 ml

HAMA blocker

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

Description	Cat.#	Qty
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)

Normal Sera and IgG from several species Immunoglobulins and serum proteins

These reagents are prepared from blood of healthy animals. They are typically used as controls or as additives in various immuno-techniques.

Applications :

Normal sera and Immunoglobulins are used for :

- ◆ Protein controls and standards for assays or analysis
- ◆ Standardized samples (i.e. serum containing 10 µg/ml of a standard hormone)
- ◆ Dilution of blood samples (dosage at constant protein concentration...)
- ◆ Saturation of tubes, vials, gels, columns, or filters to prevent non-specific adsorption of diluted molecules
- ◆ Stabilization of reagents
- ◆ Saturation of IgG binding sites for immuno-reagents (IgG receptors of cells in FCM, IF and IHC)
- ◆ Equilibrium studies (free and bound fractions of a drug in serum)
- ◆ Co-precipitation: normal IgGs are added to precipitate a diluted monoclonal
- ◆ Living cells : suspension media for cells, opsonisation of bacteria...
- ◆ Immunological studies : natural antibodies, auto-immune antibodies, immune complexes...
- ◆ Purification of blood components

◆ Normal Serum

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

◆ Normal pure IgG

Immunoglobulins from class G (IgG) have been purified from serum to achieve a purity greater than 90% (checked by electrophoresis). IgG concentration is determined by spectrophotometry. Uptima Pure IgG's are solubilised at 5 mg/ml in PBS buffer with 0.09% of sodium azide, and filtered using a 0.45 µm filter.

	Normal Serum		Normal purified IgG	
Bovine	UP89243C	2 ml		
	UP89243A	10 ml		
	UP89243B	100 ml	UP757700	15 mg
Cat	989140	5 ml	869310	10 mg
Chicken	UP37908A	10 ml	773320	25 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	15 mg
GuineaPig	UP37916A	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	15 mg
Mouse	UP379120	2 ml		
	UP379121	10 ml	UP386670	5 mg
Rabbit	UP37906B	5 ml	UP378416	15 mg
Rat	UP37911A	2 ml		
	UP37911B	10 ml	UP443086	5 mg
Sheep	UP697927	10 ml	UP797560	15 mg
Swine	UP379021	10 ml		
	UP379022	100 ml	UP062701	15 mg