Blocking solutions

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Background staining

Nonspecific staining appears in immunohistological preparations in a multifactorial way and in variable degrees. Because the so-called "background" reactions often affect the interpretation of results, appropriate controls are necessary to recognize the underlying reasons (for details see chapters *Artefactual staining in immunohistology* and *Troubleshooting guide*).

Apart from natural antibodies (being present in individuals prior to immunization), cross-reacting antibodies, Fc receptors etc. which can be the reason for non-specific immunostainings, various other factors are responsible for background reactions. This has to be carefully controlled in the material under study. In order to solve background problems at least to some extent, blocking solutions can be employed.

For routine work, a variety of blocking solutions have been formulated which indeed enable reduction of some major causes, f.e.

- Reduction of hydrophobic binding of immunoglobulins with tissues:
  - antibody dilution buffers with a pH different from the pI of antibodies,
  - diluents with low ionic strength,
  - addition of detergents to the diluent
  - proteins to be applied to tissue sections prior to the incubation in primary antibodies (f.e. serum or immunoglobulins of the species that provides the secondary antibodies),
  - other biomolecules such as bovine serum albumin, fish gelatin, casein etc.
- Reduction of ionic and electrostatic interactions of antibodies with tissues: use of diluent buffers with high ionic strength. Yet, it must be recognized that nonspecific staining is often the result of a combination of ionic and hydrophobic interactions
- Reduction of sulfhydryl interactions of antibodies with tissues: incubation of tissue sections with antibodies supplemented with thiol-reactive reagents such as reduced glutathione (GSH). The optimal concentration of GSH required for the immunostaining protocol needs to be determined empirically
- Blocking of endogenous enzyme activities (e.g. peroxidases, alkaline phosphatases): inhibition of natural enzyme activities present in tissue cells
- Blocking of avidin and biotin interactions: measures to prevent the interaction of highly charged avidin (in the detection reagents) with oppositely charged cellular molecules. Similarly, prevention of binding of endogenous biotin with avidin. Both nonspecific reactions can be partially avoided by preincubation with free avidin or biotin or by incubation in "avidin-biotin detection reagents" which are prepared at high pH (f.e. pH 9) or by the addition of non-fat dry milk (5%). Instead of avidin from egg white, the use of streptavidin from *Streptomyces avidinii* is strongly recommended to avoid nonspecific binding as much as possible.

Typical blocking solutions*

Blocking action	Reduction of background	Notes
Ionic strength of buffers, detergents	Saline and detergents added to phosphate or Tris buffers: (a) Low or high ionic strength by addition of NaCl (b) 0.01-0.5% Tween 20	Reduction of ionic and hydrophobic interactions

Blocking action	Reduction of background	Notes
Serum proteins, gelatin detergents	Use of serum from the species that provides the secondary antibodies: (a) 2-5% Sheep normal serum *plus* 1% BSA and 0.1% cold fish gelatin in 0.01 M PBS (phosphate buffered saline) (b) 2-5% Goat normal serum *plus* 1% BSA and 0.1% cold fish gelatin in 0.01 M PBS (phosphate buffered saline) (c) 2-5% Rabbit normal serum *plus* 1% BSA and 0.1% cold fish gelatin in 0.01 M PBS (phosphate buffered saline) (d) 2-5% Swine normal serum *plus* 1% BSA and 0.1% cold fish gelatin in 0.01 M PBS (phosphate buffered saline) (e) Detergents can be optionally added, f.e. 0.05% Tween 20 or 0.1% Triton X-100	Reduction of hydrophobic binding and other protein-protein interactions

* Blocking solutions can be toxic. They must be handled with care

Blocking action	Reduction of background	Notes
Dry milk, casein detergents	Milk proteins and detergents added to the buffers: (a) 0.1-5.0% Non-fat dry milk or 0.1-2% casein (b) Detergents can be optionally added, f.e. 0.05% Tween 20 or 0.1% Triton X-100	Reduction of hydrophobic binding and other protein-protein interactions

Blocking action	Reduction of background	Notes
Endogenous enzymes	Inhibition of enzyme activity: (a) Peroxidase inhibition with 1-2% H_2O_2 in PBS, block sections for 30 min (b) Peroxidase inhibition with 0.3% H_2O_2 in PBS plus 0.1% sodium azide (b) Peroxidase inhibition with methanol followed by 0.03% H_2O_2 in PBS block sections for 20 min in each solution, respectively (c) Peroxidase inhibition with 7.5% H_2O_2 for 5 min, then 2.28% periodic acid for 5 min, and finally by 0.02% sodium borohydride for 2 min (d) Peroxidase inhibition with 1% sodium nitroferricyanide in absolute methanol containing 0.2% acetic acid (d) Alkaline phosphatase (AP, nonintestinal form) inhibition with 1 mM levamisole added to the substrate mixture	Inhibition of endogenous peroxidases; the type of blocking solution will depend on the antigen and the tissue under study as well as on the type of tissue preparation. The time needed for enzyme blocking can vary and depends on the tissue type, the concentration of the blocking substance and the diluting medium Inhibition of endogenous alkaline phosphatases (AP); the AP isoenzyme from calf intestine used as label in immuno-alkaline methods is not inhibited

Blocking action	Reduction of background	Notes
Avidin and biotin	Prevention of interaction of oppositely charged molecules; preincubation with unlabeled avidin and biotin:	Blocking of charged avidin to react with tissue and prevention of binding of avidin with

	(a) use of buffers with high pH for the avidin-biotin reagents (b) preincubation with 0.01-0.1% avidin and 0.001-0.01% biotin (c) use of streptavidin instead of avidin	endogenous biotin

More details concerning *Washing Solutions* and *Incubation and antibody dilution buffers* are given in the respective chapters.

Selected publications for further readings

Grossi CE and von Mayersbach H (1964)
Streefkerk JG (1972)
Straus W (1972, 1974)
Kuhlmann WD (1975)
Kuhlmann WD (1978)
Hall JG *et al*. (1978)
Laurila P *et a*l. (1978)
Kuhlmann WD (1979)
Heyderman E (1979)
Kuhlmann WD and Krischan R (1981)
Wood GS and Warnke R (1981)
Buchmann A *et al*. (1985)
Duhamel RC and Johnson DA (1985)
Li CY *et al*. (1987)
Tacha DE and McKinney LA (1992)
Kuhlmann WD and Peschke P (2006)
Rogers AB *et al*. (2006)

Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

 10.06.2007