Incubation and antibody dilution buffers

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Primary and secondary antibodies, conjugates and the various detection systems must be made up and diluted in appropriate solution systems before they can be applied in histological immunostaining. Hence, incubation and antibody dilution buffers serve the same purpose.

Phosphate buffered saline and Tris buffered saline are the most often used media. Optionally, these can be supplemented with blocking and stabilizing molecules such as bovine serum albumin (BSA) in order to prevent background reactions. When diluted antibodies have to be stored (at 4ºC), dilution buffers should also contain 0.05% sodium azide or Thimerosal as preservatives.

The best formulations for dilution of antibodies and detecting reagents (f.e. labeled secondary antibodies, avidin/Streptavidin-biotin reagents) will depend on the material under study, and this has to be selected by trial. Some possible buffer solutions are proposed below.*

Dilution buffers for immunohistological reagents

1) **Primary antibodies (conjugated or not)**
   0.01 M phosphate buffered saline (PBS) pH 7.2-7.4 is the most applied buffer system in routine work; the use of buffers with molarities up to 0.1 mol/L phosphate have been also described.

   The addition of so-called *background-blocking* substances is often practiced as described in many papers. Dilution buffers are supplemented by those blocking substances in order to reduce background reactions. A variety of possibilities exist for this purpose; for details see chapter *Blocking solutions*:
   (a) 0.01 M phosphate buffered saline (PBS) pH 7.2 supplemented with
   1% bovine serum albumin (BSA)
   (b) 0.01 M phosphate buffered saline (PBS) pH 7.2 supplemented with
   1% BSA and normal serum (non immune serum up to 5% final)
   (c) 0.01 M phosphate buffered saline (PBS) pH 7.2 supplemented with
   1% BSA and
   0.1% cold fish gelatin and
   0.05% sodium azide (for peroxidase labeled reagents use Thimerosal instead of azide)
   (d) any other buffer described in the chapter *Washing solutions* may be used

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

2) **Secondary antibodies**
0.01 M phosphate buffer saline (PBS) pH 7.2-7.4 supplemented with
0.05% sodium azide (for peroxidase labeled reagents use Thimerosal instead of azide)

3) **Peroxidase-Streptavidin detection system**
0.01 M phosphate buffer saline (PBS) pH 7.2-7.4 supplemented with
0.05% Thimerosal
Note: do not use solutions which contain serum in order to dilute avidin-streptavidin conjugates because serum may contain biotin. The latter will reduce reaction activity of avidin-streptavidin conjugates

4) **Alkaline Phosphatase-Streptavidin detection system**
0.05 M Tris-HCl buffered saline (TBS) pH 7.6 supplemented with
1 mM levamisole and
0.05% Thimerosal

Numerous manufacturers offer “universal kits” or a variety of immunohistological reagents including buffer systems, blocking solutions, primary (conjugated or not), secondary antibodies and detection reagents such as PAP complexes and avidin-biotin systems. Many of these kits and reagents work well, but it is important to mention that each laboratory should determine their own ways of immunostaining.

Selected publications for further readings

Hall JG *et al*. (1978)
Kuhlmann WD (1978)
Laurila P *et al*. (1978)
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)
Kuhlmann WD and Peschke P (1985)
Tacha DE and McKinney LA (1992)
Ramos-Vara JA (2005)

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

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