Washing solutions

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Between the various incubation steps, histological preparations (sections, cytospins, cell cultures etc.) must be treated by washing solutions in order to take off unreacted substances which might interfere with reagents added in subsequent stages. The type of washing as well as the type of appropriate washing solution will depend on the material under study. In the early days of immunohistology with fluorescent labeled antibodies (COONS AH et al. 1942), washing steps consisted simply in the use of water or saline (0.9% sodium chloride). Also, distilled water supplemented with detergents (e.g. Tween 20) can be satisfying. The nowadays employed buffer solutions became introduced much later.

The development of balanced salt solutions dates back to the work of S RINGER (1885) who used a solution of inorganic salts to maintain viable cells in culture. Since that time, many other balanced salt solutions have been developed. Phosphate buffered saline (PBS) can be regarded as a direct derivative from this research activity. PBS is a simple buffer formulations and most often employed for the bulk of washing steps in immunohistology. The molarity of phosphate buffer may vary from 0.01 to 0.1 M phosphate salts with pH in the range of 7.2 to 7.6.

PBS has many uses because it is isotonic and non-toxic to cells. A variety of supplements can be added and used for incubation or dilution of reagents such as antibodies. Nevertheless, quite often individualized procedures can be necessary to obtain optimal immunostainings.

From the number of buffer formulations described in the chapter *Buffer solutions*, we give here a selection of the most applied washing buffers for routine immunohistology. The buffer solutions are rapidly prepared.[*]

Typical washing solutions

Washing buffer	**Buffer composition**
Phosphate buffer	**Phosphate buffer 0.1 M pH 7.2** Na_2HPO_4 (anhydrous) 10.9 g NaH_2PO_4 (anhydrous) 3.2 g distilled water ad 1000 mL Mix and adjust pH to 7.2. Dilute 1:10 with distilled water prior to use and adjust pH if necessary
Phosphate buffered saline (PBS)	**PBS 0.1 M pH 7.2** Na_2HPO_4 (anhydrous) 10.9 g

[*] Washing solutions can be toxic. They must be handled with care

	NaH_2PO_4 (anhydrous) 3.2 g NaCl 90.0 g distilled water ad 1000 mL Mix and adjust pH to 7.2. Dilute 1:10 with distilled water prior to use and adjust pH if necessary
Phosphate buffer supplemented with 0.5 M NaCl and 1% bovine serum albumin	**Phosphate buffer 0.1 M pH 7.2 plus 0.5 M NaCl and 1% BSA** Na_2HPO_4 (anhydrous) 10.9 g NaH_2PO_4 (anhydrous) 3.2 g NaCl 90.0 g distilled water ad 1000 mL Mix and adjust pH to 7.2 (dilution 1:10 with distilled water prior to use is optional, adjust pH if necessary), supplement with NaCl (0.5 M final) and bovine serum albumin (BSA, 1% final) and adjust pH
Phosphate buffered saline (PBS)-Tween 20	**PBS 0.1 M pH 7.2 plus 0.2% Tween 20** Na_2HPO_4 (anhydrous) 10.9 g NaH_2PO_4 (anhydrous) 3.2 g NaCl 90.0 g distilled water ad 1000 mL Mix and adjust pH to 7.2 and add 2 mL of Tween 20. Dilute 1:10 with distilled water prior to use and adjust pH if necessary

Washing buffer	**Buffer composition**
Tris-HCl buffer	**Tris-HCl 0.5M pH 7.6** Tris base* 61.0 g distilled water ad 1000 mL Mix and adjust pH to 7.6 with 2 M HCl. Dilute 1:10 with distilled water prior to use and adjust pH if necessary * Tris (hydroxymethyl) aminomethane
Tris-HCl buffered saline (TBS)	**TBS 0.5M pH 7.6** Tris base 61.0 g NaCl 90.0 g distilled water ad 1000 mL Mix and adjust pH to 7.6 with 2 M HCl. Dilute 1:10 with distilled water prior to use and adjust pH if necessary
Tris-HCl-Tween 20 buffer	**Tris-HCl 0.5M pH 7.6 plus 0.2% Tween 20** Tris base 61.0 g distilled water ad 1000 mL Mix and adjust pH to 7.6 with 2 M HCl and add 2 mL of Tween 20. Dilute 1:10 with distilled water prior to use and adjust pH if necessary
Tris-HCl buffered saline (TBS)-Tween 20	**TBS 0.5M pH 7.6 plus 0.2% Tween 20** Tris base 61.0 g NaCl 90.0 g distilled water ad 1000 mL Mix and adjust pH to 7.6 with 2 M HCl and add 2 mL of Tween 20. Dilute 1:10 with distilled water prior to use and adjust pH if necessary

Selected publications for further readings

Ringer S (1885)
Tyrode MV (1910)
Coons HA *et al*. (1942)
Dulbecco R and Vogt M (1954)
Kuhlmann WD *et al*. (1970)
Kuhlmann WD (1978)
Kuhlmann WD and Krischan R (1981)
Buchmann A *et al*. (1985)
Rose NR et al. (1986)
Weir DM et al. (1986)
Harlow E and Lane D (1988)
Kuhlmann WD and Peschke P (2006)

Full version of citations in chapter *References*.

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