

# B is for Bluing

**A-Z of Staining** - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.



The process of bluing is to convert the soluble red component of haematoxylin into an insoluble blue. It is a pH dependent reaction occurring in an alkaline solution. As haematoxylin is responsible for nuclear staining, the nucleus is therefore stained blue (or blue-purple) in standard haematoxylin and eosin staining.

The bluing step can cause difficulties in the lab. If underperformed, the colours of the nuclear stain will be abnormal which may cause concern or confusion. It is not possible to over-blue a section as the bluing reagent can only turn blue

the amount of haematoxylin in the tissue however, excessive bluing can cause section to lift due to the alkaline action on the tissue. If the section is too blue, this can mean there is too much haematoxylin in the section. Alternatively, the section may have dense clusters of nuclei which have all been correctly stained blue- as found in lymph nodes. Since the bluing reaction has a specific end point, the timing of the bluing step can easily be determined visually when the tissues turn 'blue'.

There are several bluing reagents available which may be used in the lab. Reagents include ammonia solutions, tap water, [Scott's Tap Water Substitute](#) (TWS), and lithium carbonate solutions. The type of water in your area may influence your choice of reagent. Tap water is only suitable for use in hard water areas whereas Scott's TWS is more suitable in areas where water is 'soft' or acidic. It is also important to be aware that sections may fall off if left too long in a harsh bluing agent, such as an ammonia solution. With dilute aqueous solutions (e.g. water, Scott's TWS) no more than one minute is required.

One example of a problem which may be caused during the bluing process is a transient effect- sometimes called 'Bluing Artefact.' When this effect occurs, nuclei and associated cytoplasm appear pale blue and look 'washed out.' The cause for the sudden appearance and subsequent disappearance of this effect has long been debated, with one user finding the source of the issue at their lab lay at a water treatment plant. The treatment plant had decided to add extra fluoride to the water resulting in different staining characteristics. In this instance, the user switched to bluing in Scott's TWS instead of tap water.

Do you experience problems with bluing in your lab? Have you noticed any differences between different tissue types? Does tap water work for you? Get in touch and let us know!

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