

W is for Weigert's stain

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.



Welcome to the ABC of Staining series where we continue with the letter 'W' for Weigert's stain

Weigert's stain is a histochemical stain used to differentiate tissue structures such as elastic fibres and myelin. The stain involved a combination of reagents for two main applications, namely iron haematoxylin for staining nuclei and myelin, and Weigert's elastin stain for identifying elastic fibres.

Weigert's iron haematoxylin is an acid-resistant nuclear stain that is used to stain cell nuclei dark purple to black. The solution is prepared using an iron salt (such as ferric chloride) as a mordant. As a result, the haematoxylin becomes oxidized to haematein which combines with the ferric chloride to form a stable iron-haematein complex. As the solution resists decolourization better than other

haematoxylin solutions, it is commonly used in prolonged special staining methods such as the trichrome stains. Weigert's stain is typically prepared from two separate components that are mixed immediately before use. Solution A comprises alcoholic haematoxylin solution whilst solution B contains the iron salt which acts as both an oxidizer and a mordant. When staining for collagen, the iron haematoxylin is often combined with a Van Gieson counterstain which uses an acid-resistant nuclear stain to contrast the blue-black nuclei from the red collagen fibres.

When staining myelin, the iron haematoxylin can be used as a regressive stain by deliberately overstaining tissue components. Under microscopic control, sections can have excess stain removed with a differentiating solution such as iron alum or an acid alcohol solution. In this way, the overstaining of unwanted areas can be removed to leave desired structures such as myelinated and non-myelinated structures strongly stained against a paler background. Weigert's stain is often used with a counterstain such as eosin which distinguishes the black myelin from other components which stain pink or red. Myelin can also be stained using the Weigert-Pal modification which utilizes the regressive nature of iron haematoxylin followed by removal of excess stain by differentiating in Pal's solution. This ultimately bleaches the grey matter to leave the myelin staining a dark blue to black due to the formation of an iron-haematoxylin complex that binds to the fatty myelin. However, the technique has proved to be time consuming and has been replaced by more improved methods (see Luxol Fast Blue entry).

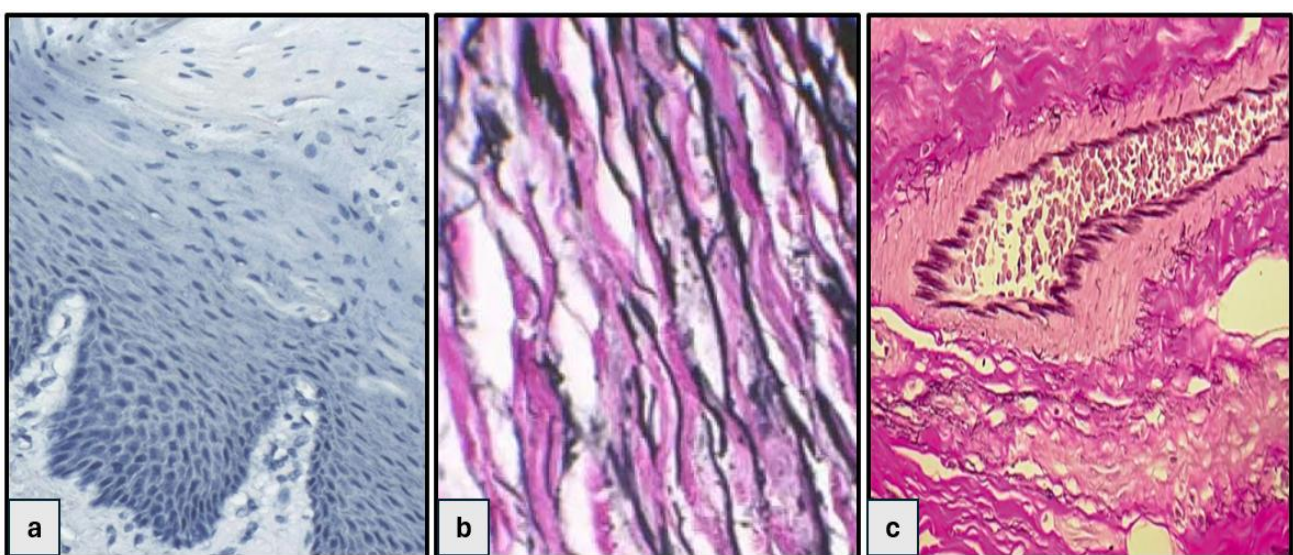


Figure. Weigert's iron haematoxylin staining showing blue-black nuclei (a), elastic fibres with Van Gieson counterstain (b), and elastic fibres differentiated with resorcin-fuchsin

The staining of elastin using Weigert's method also involves a stain combination dedicated to specifically stain elastic fibres. Dyes such as orcein, resorcin and fuchsin are often used where elastic fibres appear blue to black against a nuclear fast red or haematoxylin counterstain. Because Weigert's iron haematoxylin is resistant to the acidic conditions of many staining methods, it has become a reliable choice for staining elastic fibres.

Further reading

Dey P (2022). Haematoxylin and Eosin Stain of the Tissue Section. In: Basic and Advanced Laboratory Techniques in Histopathology and Cytology. Springer, Singapore. doi: [10.1007/978-981-19-6616-3_8](https://doi.org/10.1007/978-981-19-6616-3_8)

Dey P (2022). Connective Tissue Stain: Principle and Procedure. In: Basic and Advanced Laboratory Techniques in Histopathology and Cytology. Springer, Singapore. doi: [10.1007/978-981-19-6616-3_10](https://doi.org/10.1007/978-981-19-6616-3_10)

Kiernan J (2015). Histological and Histochemical Methods, Fifth edition: Theory and Practice. Scion Publishing Ltd, UK. ISBN-10: 1907904328 / ISBN-13: 978-1907904325

Sammet K (2008). Carl Weigert (1845-1904). Journal of Neurology 2008; 255:1439-1440. doi: [10.1007/s00415-008-0917-4](https://doi.org/10.1007/s00415-008-0917-4)

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