

J is for Janus Green

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.

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The ability to stain living cells without killing them is termed vital staining. Whether the cells are removed (supravital) or remain in the body (intravital), the terms are interchangeable and often simply referred to as vital staining. Vital stains have been used in diagnostic and surgical techniques and in this article, we take a look at Janus Green B whose chemical structure is shown below (Figure 1). Like many other certified dyes, Janus Green B is known by other synonyms such as Diazin Green S and Union Green B.

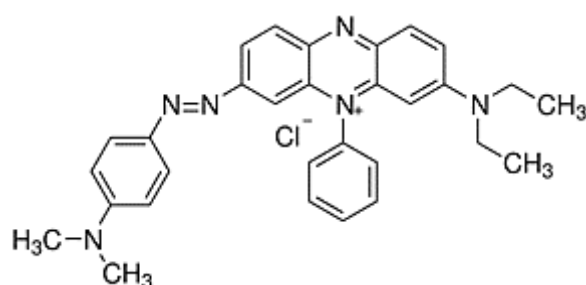


Figure 1. Chemical structure of Janus Green B (C₃₀H₃₁N₆.Cl)

Janus Green B is a dark green to black powder belonging to the phenazine group of dyes. Introduced by Leonor Michaelis in 1900, the interesting feature of this dye is that it must be oxidized to become coloured. In histology, this basic dye is used to stain mitochondria in living tissues. Regarded as the power house of cells, mitochondria are membrane-bound organelles that produce adenosine triphosphate (ATP), the primary carrier of energy. It was Michaelis in 1900 who first selectively stained these organelles with Janus Green B and proposed that mitochondria were cellular oxidizing agents. The specificity of this stain for mitochondria is due to the activity of cytochrome oxidase which maintains the dye in its oxidised, blue-green state (Figure 2). In the rest of the cell, the dye is reduced to a pink or colourless compound. Studies of lymphatic vessels in rabbits have also shown that the dye highlights the threadlike structures known as Bonghan ducts since they contain a high density of mitochondria. When stained with Janus Green B and viewed under dark ground microscopy, mitochondria appear as bright orange-yellow granules (Figure 3).

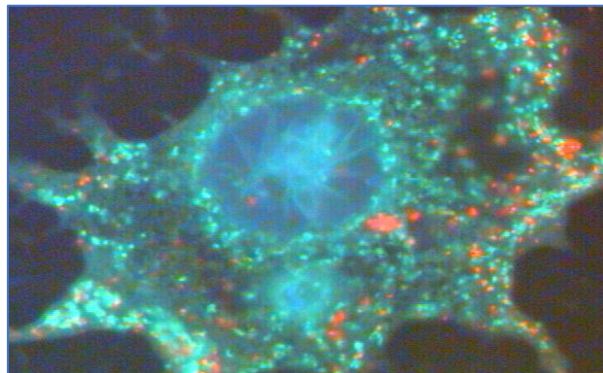


Figure 2. Hamster ovarian cell with mitochondria staining blue-green
(Courtesy of CL Case, <https://accounts.smccd.edu/case/biol230/ex3.html>)

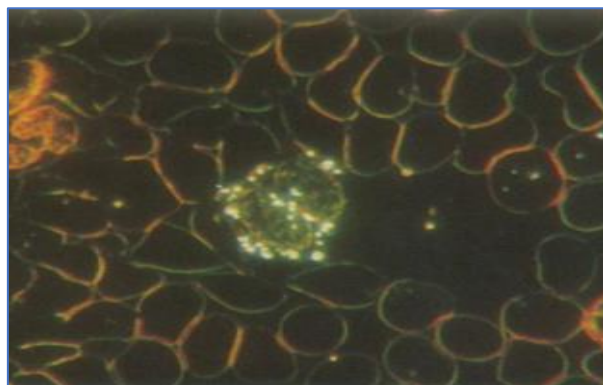


Figure 3. Mitochondria are seen as bright orange-yellow granules when viewed under high power with dark ground microscopy (Todd & Barnetson, 1988)

Additionally, Janus Green B can be used to stain other living material such as fungi, yeast cells, chromosomes and nucleic acids. When the dye is used in the presence of oxygen within the tissues, the indicator dye oxidizes to the blue-green colour. The use of Janus Green B has proved so useful as a flagella stain that it has been applied to both protozoa and spermatozoa (Figure 4).



Figure 4. Spermatozoa stained with Janus Green B (Aksoy et al, 2012)

In the laboratory, other uses of Janus Green B have included assays for the diagnosis of amyloid-related diseases and the rapid staining of peripheral nerves of insects.

Further reading

Ahmad F, Alamoudi W et al (2018). Simple, reliable, and time-efficient colorimetric method for the assessment of mitochondrial function and toxicity. *Bosnian Journal of Basic Medical Sciences* 2018;18(4):367-74

<https://www.bjbms.org/ojs/index.php/bjbms/article/view/3323>

Aksoy E, Aktan M et al (2012). Assessment of spermatozoa morphology under light microscopy with different histologic stains and comparison of morphometric measurements. *International Journal of Morphology* 2012;30(4):1544-1550

https://www.researchgate.net/publication/286727438_Assessment_of_spermatozoa_morphology_under_light_microscopy_with_different_histologic_stains_and_comparison_of_morphometric_measurements

Kudo K, Suemoto T et al (2000). Azure A analogs as imaging agents and probes for diagnosis of diseases related to amyloid. *Japanese Kokai Tokkyo Koho JP 2000344685*, 2000.

https://www.researchgate.net/publication/291293109_Azure_A_analogs_as_imaging_agents

[ts and probes for diagnosis of diseases related to amyloid accumulation Jpn Kokai Tokkyo Koho JP 2000344685 2000](#)

Todd AS & Barnetson WK (1988). Use of dark ground microscopy in haematology. Journal of Clinical Pathology 1988;41:786-792

<https://jcp.bmj.com/content/jclinpath/41/7/786.full.pdf>

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