

S is for Sirius Red

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 'S' for the dye Sirius Red. Primarily used to demonstrate collagen and amyloid, Sirius Red F3B is a hydrophilic, azo dye that is also known as Direct Red 80 (Figure 1). Sirius Red binds specifically to collagen and distinguishes type I (the most common type found in many tissues and organs) and type III fibres (found in skin and muscle). Type II collagen is mainly found in cartilage. Sirius Red F3B is not to be confused with Sirius Red 4B which has the molecular structure $C_{29}H_{19}N_5O_8S_2Na_2$ and colour index number 28160. Also known as Direct Red 81 (and Chlorantine Fast Red), Sirius Red 4B can also be used as a substitute for acid fuchsin in the

van Gieson method for staining collagen. However, unlike Sirius Red F3B, it is not suitable for demonstrating amyloid.

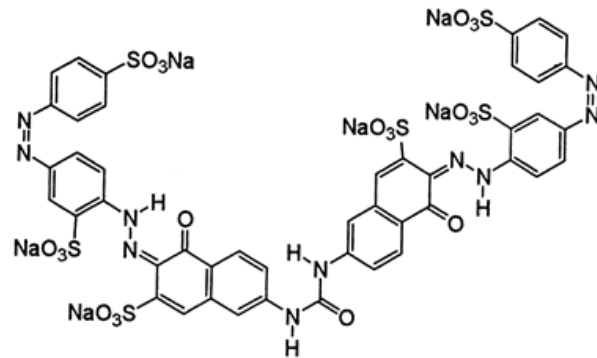


Figure 1. Sirius Red F3B (Direct Red 80) has the molecular structure $C_{45}H_{26}N_{10}O_{21}S_6Na_6$ and colour index number 35780

An established method for demonstrating deposits of collagen is the Picro-Sirius Red stain (PSR), widely used as a variant of van Gieson's stain where the dye Sirius Red F3B is substituted for acid fuchsin. This method has a high affinity for collagen which is visualized as bright yellow to orange fibres when sections are viewed under polarized light (Figure 2). Although this is the preferred technique to visualize collagen deposits, the Masson's Trichrome can equally be applied. However, this method has often been found to be less specific and sensitive than the PSR technique when demonstrating collagen.

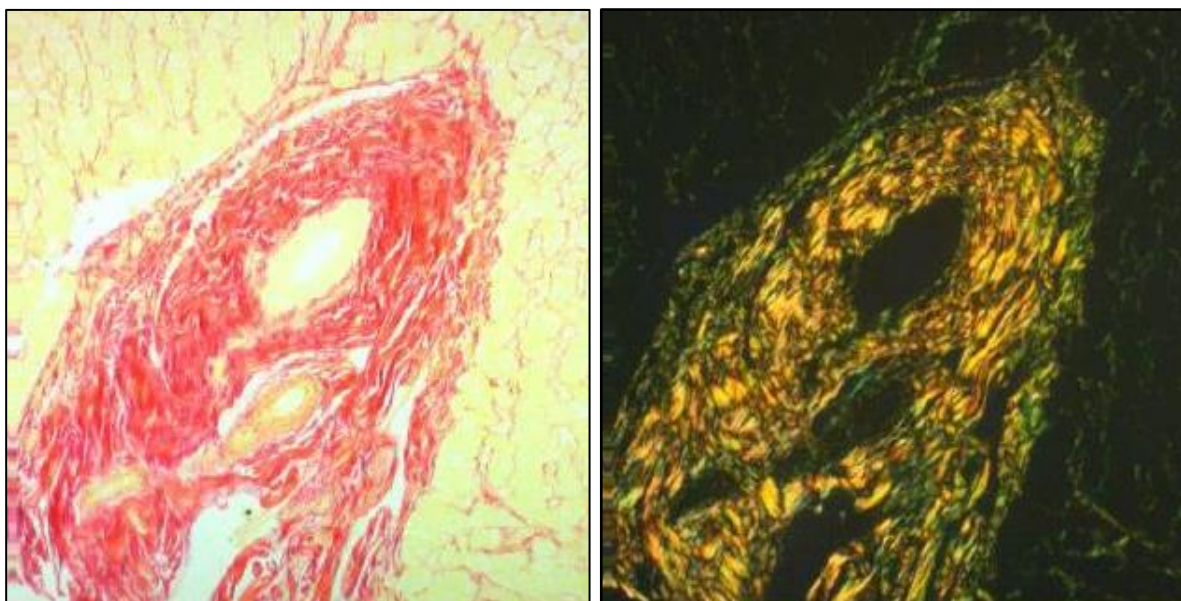


Figure 2. Collagen stained red with PSR (left) showing yellow to orange fibres when viewed under polarized light (right)

In disease processes, evaluation of collagen deposits in tissue sections is critical for the grading and staging of fibrotic changes. Fibrosis is a pathological manifestation that results in excessive collagen deposition which can play a significant role in both the growth and progression of tumours. Consequently, early diagnosis of disease processes with accurate staging of fibrosis using PSR or equivalent staining is essential. Differential staining of collagen and non-collagenous proteins can also be applied by using the dyes Picro-Sirius Red and Fast Green in combination (PSR/FG). This method has been found to be more sensitive than using van Gieson or Sirius Red alone in detecting collagen fibres. Fibrosis evaluation of red collagen is easily distinguished from non-collagenous proteins which stain green with the Fast Green dye (Figure 3).

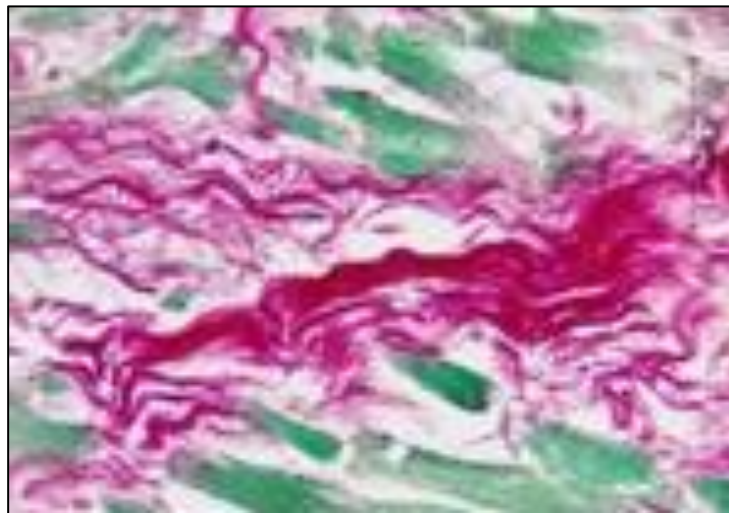


Figure 3. Section stained with PSR/FG showing thickened, red collagen fibres and green non-collagenous proteins

Sirius Red F3B is a dependable stain for the detection of amyloid deposits and tissue sections suspected of involvement by amyloidosis must be examined under both light and polarizing microscopy. With this stain, amyloid appears as crimson deposits with light microscopy which shows apple-green birefringence when viewed under polarized light (Figure 4). However, the gold standard for confirming a positive reaction for amyloid is the diazo dye Congo Red. This method is generally preferred over Sirius Red since Congo Red has been found to have greater specificity and sensitivity for staining amyloid. Nonetheless, a staining method that combines both Sirius and Congo Red has been described for demonstrating amyloid in plastic-

embedded tissue. With this combination stain, amyloid stained brighter and with better contrast than that seen with methods employing Congo Red alone.

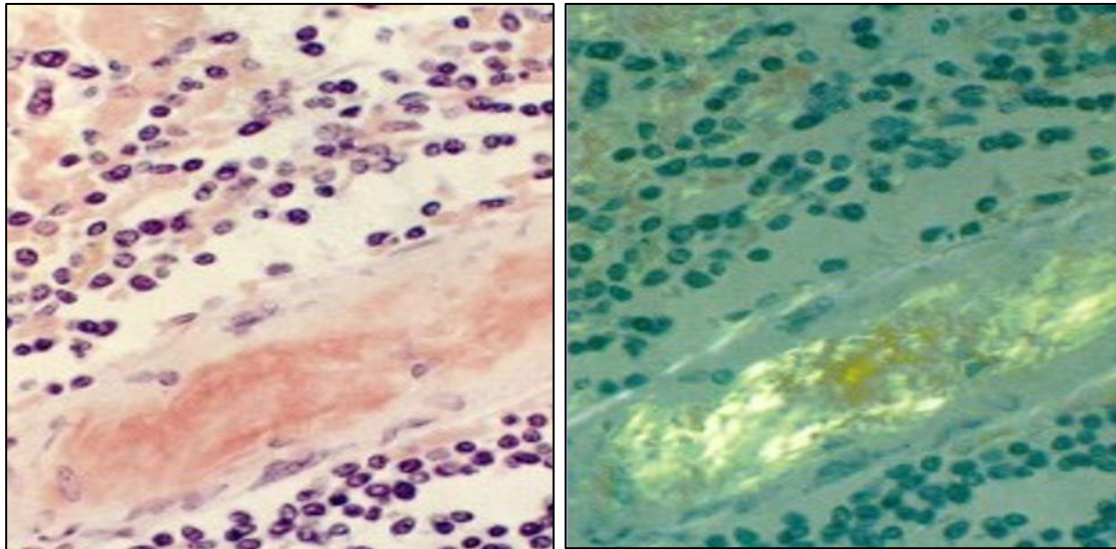


Figure 4. Amyloid staining red with PSR (left) and showing apple-green birefringence under polarized light (right)

The positive green birefringence that is characteristic of amyloid in both Congo and Sirius Red-stained sections can still be observed with this combination stain. However, the apple-green birefringence of amyloid in these methods must not be confused with the yellow to orange birefringence of collagen that can be seen in Figure 2.

Further reading

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