

Fluorescent staining for Callose with aniline blue

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Abstract

Fluorescent staining for Callose of sieve tubes of phloem.

Calose (β -1 \rightarrow 3-glucan) is the distinguished polysaccharide present on the sieve plates, of each sieve tube member of phloem tissue. Aniline blue stain specifically callose and it is use as a marker for identification of sieve elements of phloem.

PROCEDURE - STAINING FOR CALLOSE WITH ANILINE BLUE (modification of Klaus Herburger and Andreas Holzinger (2016)).

Prepare Sørensen's phosphate buffer (0.1 M, pH=8.0) according with Ruzin (1999).

Stock solutions.

A) 0.2 M of NaH₂PO₄ (Sodium phosphate monobasic anhydrous, CAS Number: 7558-80-7) BioUltra, $\geq 99.0\%$ (T) (Sigma-Aldrich S3139).

B) 0.2 M of Na₂HPO₄ (Sodium phosphate dibasic anhydrous, CAS Number: 7558-79-4) Ultra $\geq 99.5\%$, (Sigma-Aldrich S7907).

To prepare 100 mL of the buffer (0.1 M, pH=8.0), mix 2.7 mL of stock solution (A) with 47.3 mL of stock solution (B) and adjust volume to 100 mL in a Schott flask.

Add 0.5 % Aniline blue (w/v) to freshly prepared Sørensen's phosphate buffer.

Fix tissue in 2.5% glutaraldehyde in phosphate buffer Sorensen's solution (0.1 M, pH 7.2) and processed for LR white resin embedding according with manufactures.

Obtain transverse semi thin sections (1 μ m) with an ultramicrotome and bond the sections to microscope glass slides with heat (60 C) during 15 min.

Stain sections with 0.5 % of Aniline blue (CAS Number: 28631-66-5, Sigma Aldrich, Germany) in Sørensen's phosphate buffer (0.1 M, pH=8.0) for 1 h under low light intensities ($\sim 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) to prevent degradation of the dye.

Observe tissue under an Epifluorescence Microscope (Axioscope 2 Plus, Carl Zeiss AG) using a Set 01 (excitation: band pass (BP) 365/12 nm; emission: long pass (LP) 397 nm) and connected to an Axiocam MRc5 camera. Calose of sieve elements stain bright green yellow.

References

Herburger, K., & Holzinger, A. (2016). Aniline blue and Calcofluor white staining of callose and cellulose in the streptophyte green algae Zygnema and Klebsormidium. Bio-Protocol, 6(20), e1969. <http://doi.org/10.21769/BioProtoc.1969>

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