

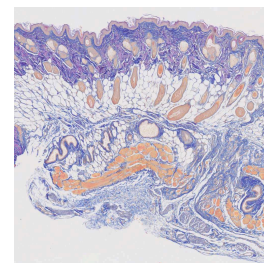
Jun 12, 2023

Version 1

AFOG V.1

DOI

dx.doi.org/10.17504/protocols.io.bp2l6xkrzlqe/v1



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DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6xkrzlqe/v1>

Protocol Citation: Joana G Antunes 2023. AFOG. [protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l6xkrzlqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l6xkrzlqe/v1)

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Protocol status: In development

We are still developing and optimizing this protocol



Created: June 12, 2023

Last Modified: June 12, 2023

Protocol Integer ID: 83248

Keywords: dyes any protein deposit, affinity for phosphomolybdic acid, amphoteric dye, phosphomolybdic acid, macromolecules of the tissue, acid fuchsin, agent between tissue structure, staining technique, dye, protein deposit, afog solution, macromolecule, tissue structure, second component of an afog solution, acid, protein, tissue, ischemic cardiac lesion, collagen, particular the regeneration, cell membrane, afog

Abstract

Staining technique largely used to diagnose ischemic cardiac lesions and kidney diseases, in particular the regeneration or scarring of the lesion. The selectivity in this method is due to the different affinity degrees between dyes and the macromolecules of the tissue. A key role is played by phosphomolybdic acid, which acts as a binding agent between tissue structures (collagen, fibrils, cell membranes) and aniline blue (amphoteric dye). Orange G, which is the second component of an AFOG solution, has no affinity for phosphomolybdic acid, and this is why it is used to stain all remaining structures. Acid fuchsin, conversely, dyes any protein deposits selectively.

Protocol materials

⊗ 1X PBS (Phosphate-buffered saline)

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⊗ Distilled Water

⊗ Bouins solution

Troubleshooting



Preparation

1h 30m

- 1 Warm the cryosections from the -80° freezer to Room temperature 00:30:00 ; 30m
- 2 If gelatin embedded tissue, incubate in 1X PBS (Phosphate-buffered saline) 00:15:00 37 °C ; 15m
- 3 Fix the slides in 4% PFA for 00:05:00 Room temperature 5m

Note

Apply the PFA directly to the slides (PFA 4% can be reused, store at 4°C)
- 4 Wash 1x in 1X PBS (Phosphate-buffered saline) 00:05:00 5m
- 5 Wash 2x in Distilled Water 00:05:00 5m
- 6 Heat Bouins solution 60 °C 00:30:00 30m

Staining

- 7 Place the slides in Bouin's solution for 2h at 60°C;
- 8 Continue to stain in Bouin's solution for an additional 1h at room temp (can be reused 3 times);
- 9 Wash slides with dH₂O 5-6 times for 5 min until the water comes out clear;
- 10 Rinse slides in 1% Phosphomolybdic acid for 5 min (can be reused 2 times);



- 11 Rinse slides in dH₂O for 5 min;
- 12 Rinse slides in AFOG staining solution for 10min (can be reused);
- 13 Rinse slides in dH₂O for 2 min;