



Immunostaining on Paraffin Sections of Fly Heads V.2

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Version 2 ▼

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1 Works for me

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ABSTRACT

This protocol describes how to perform immunostaining on paraffin sections of fly heads.

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Version created by Daniel El Kodsí

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Fix fly heads overnight in 4% formalin.

1

2 Embed in paraffin.

3 Cut 4 micron sections. Dry at \uparrow **42 °C** \circ **Overnight** .

4 Deparaffinize and bring through ethanols to water (xylene x 2, 100% ethanol x 4, tap H₂O x 2).

5 Microwave slides in [M] **10 millimolar (mM)** sodium citrate for \circ **00:15:00** .
Cool \circ **00:20:00** .

35m

Stock: [M] **100 millimolar (mM)** sodium citrate, pH 6.0

Use at least \square **1 L** of citrate solution in large glass box to avoid drying.



6 Block in PBST (PBS with 0.3% Triton) with 2% dry milk for \circ **00:30:00 to 1h**
Stock: 10X PBS

30m

7 Incubate with primary antibody \circ **Overnight** at \uparrow **Room temperature** .

8 Wash 3 x in PBST.

9 Incubate with appropriate biotinylated secondary (for immunohistochemistry) or fluorescent^{2h}
secondary (for immunofluorescence) antibody at 1:200 in PBST + milk for \circ **01:00:00** at
 \uparrow **Room temperature** .

For immunohistochemistry incubate in ABC reagent (Vector) for  **01:00:00** at  **Room temperature** .

10 Rinse 3 x PBST.

11 Mount slides with antifading medium for immunofluorescence or dehydrate through ethanol series and xylenes and mount in Permount for immunohistochemistry.