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# Immunostaining of H&E Stained Paraffin Sections of Fly Heads

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Daniel's workspace

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ABSTRACT

This protocol describes how to perform immunostaining on H&E stained paraffin sections of fly heads.

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1 Embed the fly heads in paraffin and stain with H&E for neuronal counting, per the following protocol:

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2 35m

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

Stock: [M]**100 millimolar (mM)** sodium citrate, pH 6.0
Use at least **1 L** of citrate solution in large glass box to avoid drying.

3 30m

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

4 Incubate with primary antibody **Overnight** at **Room temperature** .

5 Wash 3 x in PBST.

6 2h

Incubate with appropriate biotinylated secondary (for immunohistochemistry) or fluorescent secondary (for immunofluorescence) antibody at 1:200 in PBST + milk for **01:00:00** at **Room temperature** .
For immunohistochemistry incubate in ABC reagent (Vector) for **01:00:00** at **Room temperature** .

7 Rinse 3 x PBST.

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



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