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Immunostaining of H&E Stained Paraffin Sections of Fly Heads V.1

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We use this protocol and it's working

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Abstract

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

Troubleshooting

1 Embed the fly heads in paraffin and stain with H&E for neuronal counting, per the following protocol:
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2 Microwave slides in [M] 10 millimolar (mM) sodium citrate for 00:15:00 . 35m
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 Block in PBST (PBS with 0.3% Triton) with 2% dry milk for 00:30:00 to 1h 30m
Stock: 10X PBS

4 Incubate with primary antibody Overnight at Room temperature .

5 Wash 3 x in PBST.

6 Incubate with appropriate biotinylated secondary (for immunohistochemistry) or fluorescent secondary (for immunofluorescence) antibody at 1:200 in PBST + milk for 2h
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.