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Version 1

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

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Protocol status: Working

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:
[dx.doi.org/10.17504/protocols.io.4r3l24on4g1y/v1](https://doi.org/10.17504/protocols.io.4r3l24on4g1y/v1)
- 2 Microwave slides in [IM] 10 millimolar (mM) sodium citrate for ⌚ 00:15:00 . 35m
Cool ⌚ 00:20:00 .

Stock: [IM] 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 ⌚ 01:00:00 at 🌡 Room temperature . 2h
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.