Diaminobenzidine (DAB) Staining

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This protocol is used to stain paraffin-embedded mouse brain tissue.

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1 To paraffin-embedded tissue, deparaffinize in 100% xylenes for 3x 5 minutes. 15m

2 Incubate in 100% ethanol for 2x 5 minutes. 10m

3 Incubate in 70% ethanol for 5 minutes. 5m

4 Incubate in 50% ethanol for 5 minutes. 5m

5 Incubate in 1X PBS for 5 minutes. 5m

6 Perform antigen retrieval by incubating tissue in 1X sodium citrate for 20 minutes at 95^{o}C. 20m
Sodium citrate: 10mM sodium citrate + 0.05% Tween-20, pH6

7 Wash with 1X PBS for 5 minutes. 5m

8 Incubate in 0.9% H_2O_2 for 10 minutes at room temperature. 10m

9 Wash with 1X PBS for 5 minutes. 5m

10 Incubate in blocking buffer for 1 hour at room temperature. 1h
Blocking buffer: 10% serum + 0.05% Triton X-100 in 1X PBS

11 Wash with 1X PBS for 5 minutes. 5m

12 Incubate in primary antibody diluted in blocking buffer overnight at 4^{o}C. 1d

13 Wash with 1X PBS for 5x 5 minutes. 30m

14 Incubate in secondary antibody diluted in blocking buffer for 1 hour at room temperature. 1h

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15 Wash with 1X PBS for 5x 5 minutes. 30m

16 Incubate in streptavidin horseradish peroxidase diluted in 1X PBS for 1 hour at room temperature. 1h

17 Wash with 1X PBS for 5x 5 minutes. 30m

18 Expose to DAB for 3-10 minutes then rinse in 1X PBS for 2x 2 minutes. 15m

19 Incubate in 50% ethanol for 2 minutes. 2m

20 Incubate in 70% ethanol for 2 minutes. 2m

21 Incubate in 100% ethanol for 2 minutes. 2m

22 Incubate in 100% xylenes for 5 minutes. 5m

23 Coverslip with Permount and a #1.5 coverslip. 1m

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