

Individual Run Sheet – DAB

Bird ID: _____

Blocker: _____

Primary: _____

Secondary: _____

1. Incubate sections in **25% methanol solution** for 30 minutes at room temperature.

	Methanol	dH ₂ O	30% H ₂ O ₂
1 tray (200 mL)	50 mL	150 mL	6 mL

2. Wash sections in 1x PBS for 5x6 minutes. ☐ ☐ ☐ ☐ ☐

3. Incubate sections in **blocker** (_____) + 0.4% PBS-Tx for 1 hour at room temperature.

1 mL solution per section	Blocker	0.4% PBS-Tx
1 section	0.1 mL	1 mL
_____ sections	_____ mL	_____ mL

4. DO NOT RINSE

5. Incubate sections in **primary** (_____) (dilution 1: _____) + 0.4% PBS-Tx for _____ hours at 4°C on the shaker.

1 mL solution per section	Primary	0.4% PBS-Tx
1 section	_____ µl	1 mL
_____ sections	_____ µl	_____ mL

6. Wash sections in 1x PBS for 5x6 minutes. ☐ ☐ ☐ ☐ ☐

7. Incubate sections in **secondary** (_____) (dilution 1: _____) + blocker + 0.4% PBS-Tx for _____ hours at room temperature.

1 mL solution	Secondary	Blocker	0.4% PBS-Tx
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per section			
1 section	_____ μ l	0.025 mL	1 mL
_____ sections	_____ μ l	_____ mL	_____ mL

8. Wash sections in 1x PBS for 5x6 minutes. ☐ ☐ ☐ ☐ ☐

9. Incubate sections in **ExtrAvidin** (1:1000) + 0.4% PBS-Tx for 30 minutes at room temperature.

1mL solution per 1 section	ExtrAvidin	0.4% PBS-Tx
1 section	1 μ l	1 mL
_____ sections	_____ μ l	_____ mL

10. Wash sections in 1x PBS for 5x6 minutes. ☐ ☐ ☐ ☐ ☐

11. Prepare 4 trays with 1x PBS (20 mL each) for rinsing after DAB reaction.

12. Incubate sections in 0.025% DAB solution **without** 30% H₂O₂ for 12 minutes at room temperature.

	DAB	0.1M PBS	CoCl ₂
1 tray (200 mL)	2 aliquots (0.05g)	200mL	0.012g

Mix 0.1M PBS and CoCl₂ well before adding DAB aliquots.

13. Lift tissue out of tray and add 0.005% H₂O₂ to DAB solution, stir quickly and place tissue back into tray.

	H ₂ O ₂	dH ₂ O
1 tray (200mL)	0.060mL	6mL

14. Allow tissue to react for 30s to 90s, depending on background. Once reaction is done, immediately place in a tray with 1x PBS (200mL) for a quick rinse.

15. Wash sections in 1x PBS for 30 seconds, 1 minute, 2 minutes, and 6 minutes (x2).

30s: ☐ ☐ 1min: ☐ ☐ 2min: ☐ ☐ 6min: ☐ ☐

CLEAN ALL LAB SURFACES WITH BLEACH TO NEUTRALIZE DAB.