

General Protocol for Floating Sections-fluoro

Blocker: _____

Primary: _____

Secondary: _____

Immunohistochemistry (Day 1)

1. Rinse sections 3 times with 0.1M PBS (5 min each).

4. Incubate sections for 1 hour in 0.1M PBS + 10% BLOCKING SERUM
(_____) + 0.4% Triton-X.

1mL solution per 1 section	Blocker	0.1M PBS	Triton X- 100
1 section	0.1mL	1mL	0.004mL

5. DO NOT RINSE. _____ hours in PRIMARY
(_____) (dilution _____) diluted in 0.1M
PBS + 2.5% NHS + 0.4% Triton-X (at 4°C)

1mL solution per 1 section	Primary	Blocker	0.1M PBS	Triton X- 100
1 section		0.025mL	1mL	0.004mL

Immunohistochemistry (Day 2)

6. Rinse sections 5 times with 0.1M PBS (5 min each-first rinse quick rinse).

7. 2 hours in SECONDARY (_____)
(Dilution: _____) in 0.1M PBS + 2.5% NHS + 0.4% Triton-X.

1mL solution per 1 section	Secondary	Blocker	0.1M PBS	Triton X- 100
1 section		0.025mL	1mL	0.004mL

8. Rinse sections 5 times with 0.1M PBS (5+ min each).

9. Mount

Individual Run Sheet –Processing floating Sections

4. 1 hour in 0.1M PBS + 10% **Blocking serum** (_____) + 0.4% Triton-X.

1mL solution per 1 section	blocker	0.1M PBS	Triton X- 100
1 section	0.1mL	1mL	0.004mL
_____ sections	_____mL	_____mL	_____mL

5. _____ hours in primary: **Primary** (_____)
diluted in 0.1M PBS (performed at 4°C).

Primary Dilution: _____, Time Incubated _____.

1mL solution per 1 section	Primary	blocker	0.1M PBS	Triton X-100
1 section	_____µl	0.025mL	1mL	0.004mL
_____ sections	_____µl	_____mL	_____mL	_____mL

7. 2 hours for secondary antibody: **Secondary** (_____)
in 0.1M PBS + 2.5% NHS + 0.4% Triton-X.

Secondary Dilution: _____, Time Incubated _____.

1mL solution per 1 section	Secondary	blocker	0.1M PBS	Triton X-100
1 section	_____µl	0.025mL	1mL	0.004mL
_____ sections	_____µl	_____mL	_____mL	_____mL