Multiplex Labeling with Tyramide Fluorophores (Free-Floating Tissues)-Killinger Lab 2024

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APR 02, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.yxmvme7zng3p/v1

Protocol Citation: Bryan Killinger, Solji_G_Choi, Tyler_Tittle 2024. Multiplex Labeling with Tyramide Fluorophores (Free-Floating Tissues)-Killinger Lab 2024. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvme7zng3p/v1

Protocol status: Working
We use this protocol and it's working

ABSTRACT

This protocol details the multiplex labeling of free-floating tissues using tyramide fluorophores in the killinger lab (2024).

ATTACHMENTS

2024 Dual Labeling Using Tyramide Fluorophores.docx

Created: Mar 18, 2024

Last Modified: Apr 02, 2024

PROTOCOL integer ID: 97660

Funders Acknowledgement:
NIH-NINDS
Grant ID: 1R01NS128467
Michael J Fox Foundation
Grant ID: ASAP-024442

MATERIALS

Sodium Citrate Buffer, pH 6 **(1L):**

	A	B
	2.94 g	Sodium citrate-Trisodium salt(Dihydrate)in 1000 mL DI water.
	6.0 pH	Adjust pH to
	0.5 mL	Tween-20 (Mix well)

Blocking buffer:

	A	B
	100 mL	Dilution media
	3 mL	Normal serum
	2 g	Bovine serum albumin
	0.4 mL	Triton x100 (Mix well so the Triton is completely dissolved)

[M] 0.05 Molarity (M) **Borate buffer** pH 8.5

	A	B
	300mL	DI H2O
	5.72 g	Sodium tetraborate decahydrate (P17, big bottle)

Day 1:

1h 50m

1 Wash free-floating tissue (3 x 10 minutes) in dilution media (DM).

1.1 Wash free-floating tissue for 00:10:00 in dilution media (DM) (1/3). 10m

1.2 Wash free-floating tissue for 00:10:00 in dilution media (DM) (2/3). 10m

1.3 Wash free-floating tissue for 00:10:00 in dilution media (DM) (3/3). 10m

2 Heat water bath 01:30:00 before the antigen retrieval step. 1h 30m

1. Human samples: 90 °C - 95 °C
2. Mouse samples: 80 °C - 85 °C

3 Place the dish containing sodium citrate buffer in the water bath and heat it for 00:10:00 . 10m

a. **Sodium Citrate Buffer, pH 6 (1L):**

- 2.94 g Sodium citrate-Trisodium salt (Dihydrate) in 1000 mL DI water.
- Adjust pH to 6.0.
- 0.5 mL Tween-20. Mix well.

4 Wash the tissues in sodium citrate buffer for 00:05:00 . 5m

5 Incubate the tissues in the heated sodium citrate buffer for 00:30:00 . 30m

6 Cool down the tissues by placing container in an ice bucket for 00:15:00 . 15m

7 Wash in DM for 10 minutes x 2 times.

7.1 Wash in DM for 00:10:00 (1/2). 10m

7.2 Wash in DM for 00:10:00 (2/2). 10m

8 Endogenous peroxidase inhibition and serum blocking step (01:00:00 incubation): 0.3% H_2O_2+0.1% Sodium Azide in blocking buffer. 1h

a. Blocking buffer:

	A	B
	Dilution media	100 mL
	Normal serum	3 mL
	Bovine serum albumin	2 g
	Triton x100 (Mix well so the Triton is completely dissolved)	0.4 mL

b. In 50 mL blocking buffer, add 0.5 mL 30% H_2O_2 + 0.5 mL 10% Sodium Azide.

9 Dilute primary antibody in blocking buffer. Incubate Overnight at 4 °C . 10m

Day 2:

2w 0d 2h

10 Wash (3 x 10 minutes) in dilution media.

10.1 Wash for 00:10:00 in dilution media (1/3). 10m

10.2 Wash for 00:10:00 in dilution media (2/3). 10m

10.3 Wash for 00:10:00 in dilution media (3/3). 10m

11 HRP-Secondary antibody incubation 1:1000 dilution (01:00:00). 1h

- Solvent is 100 mL DM/ 1 mL normal serum/ 1 g BSA.

12 Wash (2 x 10 minutes) in dilution media.

12.1 Wash for 00:10:00 in dilution media (1/2). 10m

12.2 Wash for 00:10:00 in dilution media (2/2). 10m

13 Wash in borate buffer for 00:10:00 . 10m

a. [M] 0.05 Molarity (M) **Borate buffer** pH 8.5

	A	B
	300mL	DI H2O
	5.72 g	Sodium tetraborate decahydrate (P17, big bottle)

1. Mix well to dissolve completely.
2. Adjust to pH 8.5 .

14 Incubate with tyramide fluorophore (TF) for 00:30:00 while blocking light. 30m

a. 10 mL Borate buffer + 1 µL H_2O_2 + 5 µL TF.

15 View under the microscope to confirm successful staining.

16 Store in PBS and leave at 4 °C . It can be stored for up to 2 weeks. Otherwise, proceed with the antigen retrieval step.

Day 3: 2h 50m

17 Heat water bath 01:30:00 before the antigen retrieval step. 1h 30m

a. Human samples: 90 °C - 95 °C

b. Mouse samples: 80 °C - 85 °C

18 Place the dish containing sodium citrate buffer in the water bath and heat it for 00:10:00 . 10m

a. **Sodium Citrate Buffer, pH 6 (1L):**

- 2.94 g Sodium citrate-Trisodium salt (Dihydrate) in 1000 mL DI water.
- Adjust pH to 6.0
- 0.5 mL Tween-20. Mix well.

19 Wash the tissues in sodium citrate buffer for 00:05:00 . 5m

20 Incubate the tissues in the heated sodium citrate buffer for 00:30:00 . 30m

21 Cool down the tissues by placing container in an ice bucket for 00:15:00 . 15m

22 Wash in DM for 10 minutes x 2 times.

22.1 Wash in DM for 00:10:00 (1/2). 10m

22.2 Wash in DM for 00:10:00 (2/2). 10m

23 Endogenous peroxidase inhibition and serum blocking step (00:10:00 incubation): 0.3% H_2O_2+0.1% Sodium Azide in blocking buffer. 10m

a. Blocking buffer:

	A	B
	Dilution media	100 mL
	Normal serum	3 mL
	Bovine serum albumin	2 g
	Triton x100 (Mix well so the Triton is completely dissolved)	0.4 mL

b. In 50 mL blocking buffer, add 0.5 mL 30% H_2O_2 + 0.5 mL 10% Sodium Azide.

24 Dilute primary antibody in blocking buffer. Incubate Overnight at 4 °C . 10m

Day 4: 2h 20m

25 Wash (3 x 10 minutes) in dilution media.

25.1 Wash for 00:10:00 in dilution media (1/3). 10m

25.2 Wash for 00:10:00 in dilution media (2/3). 10m

25.3 Wash for 00:10:00 in dilution media (3/3). 10m

26 HRP-Secondary antibody incubation 1:1000 dilution (01:00:00). 1h

a. Solvent is 100 mL DM/1 mL normal serum/1g BSA.

27 Wash (2 x 10 minutes) in dilution media.

27.1 Wash for 00:10:00 in dilution media (1/2). 10m

27.2 Wash for 00:10:00 in dilution media (2/2). 10m

28 Wash in borate buffer for 00:10:00 . 10m

a. [M] 0.05 Molarity (M) **Borate buffer** pH 8.5

	A	B
	DI H2O	300mL
	Sodium tetraborate decahydrate (P17, big bottle)	5.72 g

1. It takes a while to dissolve completely.

2. Adjust to pH 8.5 .

29 Incubate with tyramide fluorophore (TF) on a shaker for 00:30:00 while blocking light. 30m

a. 10 mL Borate buffer + 1 µL H_2O_2 + 5 µL TF.

30 View under the microscope to confirm successful staining.

31 DAPI staining (00:20:00) 20m

a. 1:2000 dilution PBS. Block the light.

32 Mount the tissues on a slide, cover the slide with Fluoroshield, and coverslip. Seal with nail polish on all sides of the coverslip.

33 When the nail polish is completely dried, view under the microscope. Always protect the slides from light. Slides can be stored at 4 °C .