

Cresko Laboratory Manual

Cresko Lab

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Contents

1	The Cresko Lab	5
2	Introduction to the Lab	7
3	Mission and Vision	9
4	Expectations in the Laboratory	11
5	IACUC	13
6	Histological Protocols	15
6.1	Alizarin Staining	15

Chapter 1

The Cresko Lab

Description of our laboratory

Chapter 2

Introduction to the Lab

Description of our lab

Chapter 3

Mission and Vision

We describe our methods in this chapter.

Chapter 4

Expectations in the Laboratory

We describe our methods in this chapter.

Chapter 5

IACUC

descriptions of animal care IACUC protocols

Chapter 6

Histological Protocols

6.1 Alizarin Staining

6.1.1 PURPOSE: Alizarin staining of fixed adult stickleback.

6.1.2 MATERIALS:

- 0.5% Alizarin red S Stock: To make 50 mls add 0.25g alizarin red S powder to 50 ml water.
- 0.025% Alizarin Stain: To make 100 mls: Add 500 μ l 0.5% alizarin red S (stock) to 99.5ml 1% KOH
- 1 Liter: Add 5ml 0.5% alizarin red S (stock) to 995ml (1 liter) 1%KOH
- 3% H2O2/0.5%KOH: Mix and keep at 4C; Before using, bring to room temperature to hold down
- introducing bubbles under the skin: 0.5ml 6%H2O2 & 0.5ml 1%KOH.
- MESAB: Tricaine: 3-amino benzoic acid ethyl ester from Sigma (Cat # A-5040). Mix in fish safe container with a stir bar:
 - 400 mg tricaine powder
 - 800 mg Na2HPO4 (anhydrous)
 - 100 ml glass distilled water

Adjust to ~pH 7 with a drop at a time of 1N NaOH or 1N HCl if needed but it's usually right if you weigh the sodium phosphate carefully and measure the water with a graduated cylinder.

For storage: Aliquot into 6 x 25 ml fish safe plastic bottles and store at 4C. Label with date made and use within a couple of weeks.

8% PFA: {#pfa .subhead2}

- 8 g Pelleted PFA (Ted Pella, Inc.; cat# 18501)

- 90 ml dH₂O
 - 25 drops 1N NaOH
1. Heat at very low heat and stir until solution clears.
 2. Add 25 drops 1N HCl. pH should be 7.0-7.2.
 3. Filter and store at 4C not more than 1 week.
 4. Use as 4% PFA: dilute 1:1 with 2X PBS, do not store solution more than a few hours.

2X PBS {#x-pbs .subhead2}

- 1.6% NaCl
- 0.04% KCl
- 0.04 M PO₄ pH 7.0- 7.3

6.1.3 Procedure:

Day

Step

Time for Step

Date and Time

1

2h-8h at R/T depending on size on shaker.

1h or longer at R/T on shaker.

Without agitation and with lid open until eyes start to lighten and all skin pigment is gone (usually about an hour or more)

2

2 h to O/N at R/T on shaker

2 h to overnight O/N at R/T on shaker.

Check for bone staining.

R/T on shaker until excess stain in tissue is gone.

Without agitation

Wild caught specimens are put in 100% EtOH in the field and then rehydrated and put into 4% when back in the lab.