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

Created: Mar 26, 2020

Staining of fish Red Blood Cells

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ABSTRACT

This is a technique to extract blood and prepare blood films of fish to make stereological analysis of blood cells. This is a modification of Rosenfeld (1947) technique by Tavares-Dias and Moraes (2006) using common staining techniques and is called **May-Grünwald-Giemsa-Wright**.

[Rosenfeld, G. \(1947\) Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Memórias do Instituto Butantan 20:329-334](#)

[Tavares-Dias, M., & de Moraes, F. R. \(2006\). Características hematológicas da Tilapia rendalli Boulenger, 1896 \(Osteichthyes: Cichlidae\) capturada em "pesque-pague" de Franca, São Paulo, Brasil. Bioscience Journal, 19\(1\): 107-114.](#)

MATERIALS

MATERIALS

- ☒ Methanol **P212121 Catalog #PA-33900HPLCCS4L**
- ☒ 100ml Giemsa Stain Stock Solution **G-Biosciences Catalog #786-1065**
- ☒ 20 mg Eugenol **biorbyt Catalog #orb104769**
- ☒ Wright's stain (Eosin methyl blue) **Bio Basic Inc. Catalog #WB0989.SIZE.25g**
- ☒ 1 ml syringes (or U-100 Insulin Syringe) **BD Biosciences Catalog #329461**
- ☒ EDTA **Thermo Fisher Catalog #17892**
- ☒ Shandon™ Wright-Giemsa Stain Kit, Wright-Giemsa solution **Thermo Fisher Catalog #9990710**

STEP MATERIALS

- ☒ Ethylenediaminetetraacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E9884**
- ☒ 20 mg Eugenol **biorbyt Catalog #orb104769**

You can make your own MGGW stain solution or buy ready-to-use kits.

Last Modified: Apr 02, 2020

SAFETY WARNINGS

PROTOCOL integer ID:
34840

Methanol is volatile, so use a fume hood.

Keywords: Erythrocytes,
Blood, Blood cells

BEFORE START INSTRUCTIONS

Before collecting blood samples, you need to anaesthetize fish using a eugenol solution (50 mg /L)

20 mg Eugenol **biorbyt Catalog #orb104769**

Blood extraction

- 1 Prepare a 1 mL syringe with **[M] 3 % volume** EDTA.

Note

Syringe spec is 24 G x 3/4" (20 mm x 0.55 mm)

Ethylenediaminetetraacetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E9884**

- 1.1 Dilute 3 g of EDTA powder in 100 mL of distilled water

- 2 Extract blood from the *vena caudalis* or by cardiac puncture (for large fish specimens), or by decapitation (for small specimens)

Note

If you have small fish specimens, you don't need the syringe. You just have to use 1 µL pipette to get one drop of blood from the head of fish.

Cardiac puncture of small fish specimens

Preparing blood film

- 3 Place one blood drop on a microscope slide

Placing blood drop on the slide

- 4** Take an extra microscope slide, touch the blood drop at 45° and then slide it until the drop is fully spread

Beginning of the procedure showing how to position the extra slide

5 Let the slide dry 00:20:00

20m

Staining

6 Take about 300 μL of the MGGW stain solution and drop it on the slide so all the slide is covered.

Note

The stock solution of the May-Grünwald-Giemsa-Wright (MGGW) stain is made with 1 g of eosin methyl blue of May-Grünwald, 1 g of eosin methyl blue of Giemsa, and 1 g of eosin methyl blue of Wright, diluted in 1 L of methanol. The stock solution should be kept at 25 °C for at least 1 week before use. Then, you should filter it and it's ready to be used.

Methanol **P212121 Catalog #PA-33900HPLCCS4L**

500ml Giemsa Stain Stock Solution **G-Biosciences Catalog #786-1066**

Wright's stain (Eosin methyl blue) **Bio Basic Inc. Catalog #WB0989.SIZE.25g**

7 Wait for 00:03:00

3m

8 Add drops of distilled water to the slide with stain to dilute the stain.

Note

you can use a plastic straw or a pasteur pipette to homogenize the solution on the slide.

Expected result

A fine, shiny layer of stain precipitate will appear on the slide. This is because the stain does not dilute in methanol.

9 Wait the stain dry for 00:10:00

10m

- 10

Wash slides in tap water

00:00:15

10s
- 11

Wait slides dry

00:20:00

20m
- 12

Analyse cells in image analysis software, such as ImageJ or Motic Image Plus

Software

FIJI (Image J)

NAME

NIH

DEVELOPER

Software

Motic Images Plus

NAME

Windows and macOS

OS

Motic

DEVELOPER