

## Mouse Pathology Core

### Wright's Giemsa Staining of Slides

#### Materials

Fisher Protocol Wright Giemsa Stain (cat #: 264-984)

Fisher Protocol pH 6.8 Phosphate Buffer (cat #: 262-236)

Fisher Protocol Giemsa Stain Original Azure Blend Type (cat #: 027-273)

[No longer available. Alternative= Giemsa Stain Colorant Selon Giemsa by Harleco 620G/75 Original Azure Blend Type, it is made by EM Science, but we believe it can be obtained through Fisher]

Methanol

5 Coplin Jars

Cytoseal 60 (Fisher, cat #: 8310-4)

Fisher Premium Microscope Slides-Superfrost (cat # 12-544-7)

#### Procedure

Prepare the following Coplin Jars:

Jar 1: Fresh Methanol

Jar 2: 30 ml Wright Giemsa Stain

Jar 3: 5 ml Wright Giemsa Stain

0.4 ml Azure Blend

30 ml ddH<sub>2</sub>O

Jar 4: 3-5 ml Wright Giemsa Stain (adjust amount according to lab preference)

30 ml pH 6.8 Phosphate Buffer

Note: Upon mixing of solutions in Jars 3 and 4, an oxidate forms at the surface of the solutions. This oxidate needs to be removed for a clean staining. To do so, use an electronic pipettor fitted with a 25 ml pipette, and sample up and down (generating bubbles helps to lift the oxidate which will then attach to the walls of the pipette). Repeat this procedure until any visible masses of oxidate disappear (usually twice is sufficient). Sometimes using filter paper (GIBCO Concert High Purity Filter Paper, # 12182-010) may be useful in removing oxidate. Then place 5 microscope slides into each jar and let sit briefly. The slides should be free of oxidative debris when lifted out of the Giemsa mix. If not, dipping another set of microscope slides should be sufficient. Slides pulled out from Jar 4 should exhibit a pink blush. The stains are now ready for use.

1. Fix slides in methanol for 30 seconds. Then dab the slides onto an underpad or Kimwipe to remove excess methanol.
2. Place the slides in Jar 2 for 3 minutes. Then dab the slides onto an underpad or Kimwipe to remove excess stain.
3. Place the slides in Jar 3 for 10 minutes. Then dab the slides onto an underpad or Kimwipe to remove excess stain.
4. Place the slides in Jar 4 for 2 minutes (adjust staining time according to lab preference). Then dab the slides onto an underpad or Kimwipe to remove excess stain.
5. Place the slides into a Coplin jar containing distilled water. Agitate the jar containing the slides and then pour out the wash. Repeat this wash. Remove the slides and then briefly rinse both surfaces with distilled water.
6. Use a large Kimwipe to clean the undersurface of the slides, then place the slides under a blowing fan to air dry.
7. Coverslip with Cytoseal 60

Note: Prepare new Giemsa staining solutions weekly. Staining results may differ with different batches of Giemsa stains ordered from Fisher.