Sedona Lab Products

Quick staining Protocol: *Manual Slides*

We believe our Hemastain products represent the finest quality and best value over other commercially available Romanowsky-based stain products. Our Hemastain provides all of the brilliance of specialized stains like Wright - Giemsa, with the depth of colorization of cells and cytoplasm brilliance required for both peripheral and bone marrow smears.

Reagents:

Name	Part #	Description
Hemastain (wright's stain)	21-110	4 x 1 gallon case, Wright's Stain
Hemastain Fixative	21-210	4 x 1 Gallon case, Fixative 100% Methanol
Hemastain Buffer	21-311	20 Foil Packets, 4.4gm, 7.1pH

Reagent Preparation:

Hemastain and Hemastain Fixative is shipped ready to use in easy F style plastic bottles. The ***Buffer solution*** is prepared by using one gallon of Distilled (D.I.) Water to a single packet of our Hemastain Buffer powder. Stable at room temperature for up to 30 days. The ***Rinse Solution or Recirculating bath***: Change daily or per shift

Manual Rack Procedure:

1. Place prepped slide on staining rack or coplin jar.
2. Apply sufficient Hemastain Fixative
3. Apply sufficient Hemastain to cover the smear (leave on ~ 1 minute).
4. Add roughly the same volume of premixed buffer solution
5. Mix layers of the Hemastain / Buffer by applying a current of air or by blowing on slides (no heat). An iridescent scum should form on the slide surface in 5 to 10 seconds.
6. Allow the Hemastain / Buffer mixture to remain on the slide approximately 2 minutes.
7. Wash off Hemastain / Buffer mixture by flooding with D.I. water until the slide runs clear.
8. Remove slide from rack. Wipe back of slide and proceed to allow to air dry (low or no heat).

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Results of Procedure:

1. RBC: pink – tan color with degrees of chromasia.
2. WBC: Nuclei with bright, bluish – purple chromatin, light blue nucleoli.
    a. Lymphocyte: Clear Blue cytoplasm, red – purple granules usually present.
    b. Monocyte: Mosaic of Pink and blue cytoplasm, azure granules usually present.
    c. Neutrophils: Light purplish – pinkish or lavender granules in cytoplasm.
    d. Eosinophils: Bright red or reddish – orange granules in cytoplasm.
    e. Basophils: Deep Purple and violet – black granules in cytoplasm.
3. Platelets: Clearly demarcated red – purple granules in light blue cytoplasm.

Limitations:

A truly representative blood smear is a diagnostic tool of inestimable value to the clinician. The course of disease is often monitored by the routine Differential. Therefore, it is to be stressed that the information gathered from the blood smear is only as accurate as the preparation of the blood film, from the specimen collection, smear prep (see Autoprep), drying and finally staining of the resultant smear.

Expected Results:

The reaction of cytoplasm to neutral staining is subject to a great many variables as described above. Since the majority of staining occurs during the buffering stage (see number 3 above), the variable of greatest magnitude is the resultant pH of the Hemastain / Buffer mixture at the cellular surfaces. The overall color of the red blood cells is a guide to stain quality and should be used in adjusting the staining and buffering times for desired results. Specifically, RBC's should appear buff – pink; acid stain will render them bright red or reddish – orange, whereas alkaline staining will render them blue or green.

1. All leukocyte nuclei should appear bluish – purple. Acid stain yields pale blue and alkaline stain yields dark blue leukocyte nuclei.
2. Eosinophilic granules should appear red; Acid stain yields brilliant and distinct red granules and alkaline stain yields deep grey or blue Eosinophilic granules.
3. Neutrophilic granules should appear violet to pink; Acid stain yields pale Neutrophilic granules and alkaline stain yields dark, prominent Neutrophilic granules.
4. Lymphocyte cytoplasm should appear sky blue; Acid stain yields pale blue cytoplasm and alkaline stain yields grey or lavender Lymphocyte cytoplasm.