

Wright Stain, Buffered for Smears - Technical Memo

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|------------------------|---------------|----------------|-----------------|
| SOLUTION: | 500 ml | 1 Liter | 1 Gallon |
| Wright Stain, Buffered | Part 1422A | Part 1422B | Part 1422C |

Additionally Needed:

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|----------------------------------|------------|
| Alcohol, Methanol Anhydrous, ACS | Part 12236 |
| Wright Stain Buffer, pH 6.8 | Part 1430 |

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Wright Stain, Buffered for Smears provides a quick staining technique for differential staining of cell types in peripheral blood smears as well as bone marrow smears/films.

METHOD:

Technique: Coplin jar or flat staining rack method

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

PRESTAINING PREPARATION:

1. Prepare within an accepted time frame, a well-made blood smear or bone marrow smear/film per your laboratories protocol, with a focus on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Filter Wright Stain, Buffered prior to use with quality filter paper.
 - a. *For flat staining rack method, filter sufficient stain to allow 1 ml of stain per slide.*
4. Prepare 25% Aqueous Methanol Rinse; combine and mix well.
 - a. *Distilled Water 30 ml or 3 ml*
 - b. *Methanol (12236) 10 ml or 1 ml*

STAINING PROCEDURE:

5. **Coplin Jar Method:** See Procedure Notes #1 and #2.
 - a. *Fix smears in Methanol for 15 seconds.*
 - b. *Stain in filtered Wright Stain, Buffered for 1-2 minutes.*
 - c. *Place directly into Wright Stain Buffer, pH 6.8 (1430), for 1-4 minutes. **Do Not Agitate!***
 - d. *Dip quickly in 25% Aqueous Methanol Rinse (Step #4).*
 - e. *Rinse in distilled water.*
 - f. *Air-dry slides in a vertical position; examine microscopically.*
 - g. *If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.*
6. **Flat Staining Rack Method:** See Procedure Notes #1 and #2.
 - a. *Place slides on flat staining rack suspended over sink.*
 - b. *Fix by flooding slide with Methanol for 15 seconds.*
 - c. *Drain off Methanol.*
 - d. *Flood each slide with 1 ml of filtered Wright Stain, Buffered for 1 minute.*
 - e. *Retain Wright Stain, Buffered on slides.*
 - f. *Directly add 2 ml of Wright Stain Buffer, pH 6.8 to each slide; agitate gently to mix with retained Wright Stain.*
 - g. *Stain for an additional 3 minutes.*
 - h. *Flood smears with 25% Aqueous Methanol Rinse (Step #4) for 1 second.*
 - i. *Rinse in distilled water.*
 - j. *Air-dry slides in a vertical position; examine microscopically.*
 - k. *If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.*

RESULTS:

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|-------------------|--------------------|
| Erythrocytes | Pink |
| Neutrophils | Granules - Purple |
| Eosinophils | Granules - Pink |
| White blood cells | Chromatin - Purple |
| Lymphocytes | Cytoplasm - Blue |
| Monocytes | Cytoplasm - Blue |
| Bacteria | Deep Blue |

PROCEDURE NOTES:

1. Timings provided are suggested ranges. Optimal times will depend upon staining intensity preference.
2. Smears containing primarily normal cell populations require minimum staining time; immature cells and bone marrow smears/films may require longer staining time.
3. The color range of stained cells may vary depending on buffer pH and pH of rinse water.
 - a. *Alkalinity is indicated by red blood cells being blue-grey and white blood cells only blue.*
 - b. *Acidity is indicated by red blood cells being bright red or pink and lack of proper staining in white blood cells.*
 - c. *If necessary, adjust buffer pH accordingly to 6.8 +/- 0.2.*

REFERENCES:

1. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 747-748.
2. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-532.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 154-155.
4. Modifications developed by Newcomer Supply Laboratory.