

Differential Stain Kit, Smears & Touch Imprints - Technical Memo

KIT INCLUDES:

Part 9112B

Solution A: Xanthene Stain Solution	500ml
Solution B: Thiazine Stain Solution	500ml
Solution C: Fixative	500ml

Individual stain solutions may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Xylene, ACS Part 1445

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

APPLICATION:

The Newcomer Supply Differential Stain Kit, a modification of the Wright Giemsa Stain technique, uses aqueous based stain solutions and a methanol fixative. This stain kit provides a rapid 3-step process that can be used for differential assessment of: peripheral blood smears, touch imprints, fine needle aspirations (FNA), bone marrow biopsy aspirations, as well as detecting microorganisms.

METHOD:

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

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Prepare within an accepted time frame, a well-made blood smear, touch imprint, FNA smear or bone marrow aspiration smear/film per your laboratories protocol, with a focus on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Dip dried slides in Solution C: Fixative 5-10 times, one second per dip. Allow excess fixative to drain.
4. Dip slides in Solution A: Xanthene Stain Solution 5 times, one second per dip. Allow excess solution to drain.
 - a. See Procedure Notes #1, #2 and #3
5. Quickly rinse slides with distilled water.
6. Dip slides in Solution B: Thiazine Stain Solution 5 times, one second per dip. Allow excess solution to drain.
7. Rinse slides quickly in distilled water.
8. Allow slides to air-dry, then examine microscopically.
9. If coverslip is preferred, allow slides to air-dry; dip dried slides in xylene and coverslip with compatible mounting medium.

RESULTS:

Erythrocytes: Pink to yellowish-red
Platelets: Violet or purple granules

Granulocytes

Neutrophils: Nucleus - Dark blue to violet
Cytoplasm - Pale pink
Granules - Purple to lilac

Eosinophils: Nucleus - Blue
Cytoplasm - Blue
Granules - Red to red-orange

Basophils: Nucleus - Purple or dark blue
Granules - Dark purple

RESULTS CONTINUED:

Mononuclear Cells

Monocytes:	Nucleus – Violet Cytoplasm - Sky blue
Lymphocytes:	Nucleus – Violet Cytoplasm - Dark blue
Bacteria/microorganisms:	Deep blue in varying shapes
Muscle and collagen	Pale Pink
Nuclei	Blue/violet
Cytoplasm	Varying shades of light blue

PROCEDURE NOTES:

1. The division of stains in this kit gives the user the advantage of varying dips in Solutions A and B to produce different degrees of shading and intensity. However; never use fewer than three dips of one full second each.
2. If more intense overall stain is desired, increase the number of dips in Solutions A and B.
 - a. To increase eosinophilic staining; increase the number of dips in Solution A.
 - b. To increase basophilic staining; increase the number of dips in Solution B.
3. If a paler stain is desired; decrease dips in Solutions A and B.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for coverslipping application.

REFERENCES:

1. Bain, B.J. "Bone Marrow Aspiration". *Journal of Clinical Pathology* 54 (2001): 657-663.
2. Cox, Charles. "Accuracy of Intraoperative Imprint Cytology for Sentinel Lymph Node Evaluation in the Treatment of Breast Carcinoma." *Cancer Cytopathology* 105.1 (2005): 13-20.
3. "Guidelines of the Papanicolaou Society for Fine-Needle Aspiration Procedure and Reporting." *Diagnostic Cytopathology* 17 (1997): 239-247.
4. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-535.
5. Thompson, Samuel Wisley, and Ronald D. Hunt. *Selected Histochemical and Histopathological Methods*. 2nd ed. Springfield, IL: Thomas, 1966. 756-762.
6. Modifications developed by Newcomer Supply Laboratory.