

Thermo Scientific Richard-Allan Scientific Three-Step Stain Set Instructions for Use

**For in vitro diagnostic use.
For rapid staining of blood smears.**

Specimen Collection

Fresh blood film or fresh EDTA anticoagulated blood film and bone marrow films. Specimens should be air dried after smear has been prepared.

Technical Procedure

Immersion Staining Protocol

1. Thoroughly dry blood or bone marrow smears.
2. Pour approximately 40 mL of Fixative, Solution A, Solution B and deionized water into four separate staining jars.
3. Dip the slide in Fixative followed by Solution A then Solution B for 5-15 seconds each.
4. Allow excess to drain.
5. Finally, rinse in deionized water for 5-15 seconds.
6. Air dry smears.
7. Examine smears under a microscope.

Horizontal Staining Protocol

1. Place slide with thoroughly dried film on a horizontal staining rack.
2. Flood smear with absolute methanol for 15-30 seconds and then drain.
3. Flood smear with Fixative, Solution A and Solution B individually for 5-15 seconds. Allow excess fluid to drain between each step.
4. Rinse smear thoroughly with deionized water.
5. Air dry and examine under a microscope.

Note: To suit personal preference with regard to stain color, time can be varied between 5-15 seconds. To enhance eosinophilic staining, increase length of time in Solution A. To enhance basophilic staining, increase length of time in Solution B. For darker overall stain, increase length of time in both Solution A and Solution B. Do not return used stain to the original container as this may adversely affect the quality of the unused stain.

Results

Erythrocytes – Pale Pink
Eosinophilic Granules – Reddish Orange
Leukocyte Nuclei – Purple
Cytoplasm – Bluish Purple
Neutrophilic Granules – Light Purple

Discussion

The Thermo Scientific™ Richard-Allan Scientific™ Three Step Stain Set should be stored at room temperature. This staining reagent is for "In Vitro" use only. Refer to the Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under ordinary storage parameters. All dyes used in this formulation have been certified by the Biological Stain Commission.

Technical Comments

Thicker films and bone marrow preparations will require longer staining times. Because there is variation in the pH of tap water, use of tap water in the procedure may hinder staining results. Distilled or deionized water should be used during the staining procedure. The "ripening" of the polychromed dye is a continuous chemical reaction. Therefore, the stock solution should not be used or diluted after the expiration date. Blood films which have not been thoroughly air dried before staining may show sloughing of cells from slide. Three-Step Stain Set may also be used for fine needle aspirate and frozen section diagnosis. Immersion times may need to be adjusted to compensate for cellular thickness.

Mode of Action

The Three-Step Stain Set is comprised of three components: a methanol fixative (Fixative), an aqueous Eosin Y solution (Solution A), and an aqueous Methylene Blue/Azure A solution (Solution B). The staining solutions have anionic and cationic properties. The negatively charged phosphoric acid groups of DNA attract purple polychromatic cationic dyes to the nuclei. The blue basophilic granules are stained by the polychromatic cationic dyes. Cationic cellular components, such as erythrocytes and eosinophilic granules, are stained by the red and pink anionic dyes. The deionized or distilled water used in the staining procedure liberate and activate dye ions allowing them to chemically bond with specific cellular components. When staining blood and bone marrow smears, the pH of the staining solution and/or buffer is a critical factor.

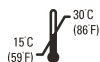
References

1. Raphael, S.S. Lynch's Medical Laboratory Technology, Fourth Edition. Saunders Company, Philadelphia, PA, 1983.
2. Lillie, R.D. H.J. Conn's Biological Stains, Ninth Edition. Williams and Watkins Company, Baltimore, MD, 1977.
3. Brown, B.A. Hematology: Principles and Procedures, Fourth Edition. Lea and Febiger Company, Philadelphia, PA, 1984.

Order Information

Product	Size	Qty.	REF
Three Step Stain Set	3x500 mL	1 set	3300
Three Step: Fixative	1 gal	1	3303
Three Step: Solution A	1 gal	1	3313
Three Step: Solution B	1 gal	1	3323

Anatomical Pathology



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