

Thermo Scientific Richard-Allan Scientific Wright-Giemsa Stain Solution Instructions for Use

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 and fixed in absolute methanol for 15 seconds.

Mode of Action

The Thermo Scientific™ Richard-Allan Scientific Wright-Giemsa Stain Solution is a mixture of several thiazin dyes in a methanol solvent. Ionic and nonionic forces are involved in the binding of these dyes. The staining solution has anionic and cationic properties. The negatively charged phosphoric acid groups of DNA attract the 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 buffers 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.

Technical Procedure

Immersion Staining Protocol

1. Thoroughly dry blood or bone marrow smears.
2. Fix smears in absolute methanol for 15 seconds to 5 minutes
3. Stain smears in Wright-Giemsa Stain Solution for 1 minute.
4. Rinse smears in Phosphate Buffer pH 6.6 solution (Catalog #89032) for 5 minutes – do not agitate slide.
5. Rinse smears briefly in running deionized water (5-6 dips).
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 Wright-Giemsa Stain Solution for 45 seconds.
4. Add equal amount of Phosphate Buffer pH 6.6 solution to smear and let stand for 1 minute.
5. Rinse smear thoroughly with Phosphate Buffer pH 6.6 solution.
6. Air dry and examine under a microscope.

Results

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

Discussion

Wright-Giemsa Stain Solution should be stored at room temperature. This staining reagent is for "In Vitro" use only. Refer to the Material Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under ordinary storage parameters. It is recommended that the Wright-Giemsa Stain Solution be discarded after each use. 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. The staining procedure may require modification to suit personal preference. Blood films which have not been thoroughly air dried before staining may show sloughing of cells from slide.

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

Ordering Information

Product	Size	Qty.	REF
Wright-Giemsa Stain	32 oz	1	89013
Wright-Giemsa Stain	1 gal	1	89014
Wright-Giemsa Stain	2.5 gal	1	89017
Phosphate Buffer pH 6.6 (powder)	9.25g	12/cs	89032

