U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Dunhua N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

TECHNICAL DATA SHEET 192

Gill's Hematoxylin - Specific for Staining Nuclei

Three formulations for flexibility in nuclear staining.

Gill's Hematoxylin No. 1 for Cytology. (Single Strength) Lower strength formulation, ideal for staining cytology.

Gill's Hematoxylin No. 2 for Histology and Cytology. (Double Strength) This intermediate formulation is used as a counterstain for immunohistochemistry (IHC) chromogens and routine Histology. It is excellent for more intense cytological staining.

Gill's Hematoxylin No. 3 for Histology. (Triple Strength) The strongest formulation of the stain provides greater intensity for histological staining of nuclei with shorter staining times.

BACKGROUND:

Cytomorphology is the study of cellular health and disease. In broad terms, examination of the cytoplasm indicates the function- al differentiation of a cell whereas observation of the nucleus can allow determination of normal or abnormal growth activity.[1] Hematoxylin is the most widely used nuclear stain. Visualizing cytomorphologic patterns is conveniently done with stained cells.

Since its successful introduction to microtechnique in 1865,[2] hematoxylin has been described in many formulations. Conceptually, a hematoxylin formulation may be categorized as a regressive or a progressive stain.[3] A progressive stain has a low hematoxylin concentration (e.g. Mayer's Hematoxylin) and slowly and selectively stains chromatin. A regressive stain has a high concentration of hematoxylin (e.g. Harris' Hematoxylin) and rapidly diffuses stain over the entire cell. Correct staining is obtained by sequentially immersing the stained material in a dilute solution of HCl in either alcohol or water to differentially extract most of the hematoxylin from the cytoplasm and some of the hematoxylin from the nucleus. For best results, the quality of differentiation should be checked microscopically before staining with another dye (for counterstaining).

MERITS OF GILL'S HEMATOXYLIN

Gill's Hematoxylin is a specially formulated solution of hematoxylin designed for use as a biological stain for the chromatin of normal and abnormal cells, whether in whole cells or sections of animal or human tissue. Following established custom, Gill's Hematoxylin takes its name from the person who first described it, Gary W. Gill.[4] Gill's Hematoxylin permits longer shelf life with greater control of staining performance and reproducibility. It stains chromatin at a controlled rate and within a narrow range of optical densities.[5-6] Therefore overstaining is less likely and differentiation in an acid solution is unnecessary. Nucleoli are delicately stained so that their acidophilia may be seen. The colors of counterstains have no interference from nuclear staining with Gill's Hematoxylin formulas.

CHEMICAL PRINCIPLES OF HEMATOXYLIN

Hematoxylin is derived from the extract of logwood and is isolated as a mixture of hematoxylin and hematein. For effectiveness as a stain, hematoxylin must be oxidized to hematein, which is then combined with a metallic iron mordant to increase the selectivity of the stain for chromatin. Sodium iodate is a convenient oxidizing agent while aluminum sulfate is the mordant.[5] Acetic acid is included in Gill's formulas to maintain pH.[7] Ethylene glycol, is included to prevent the sheen or scum from forming, unless it is exposed to air in a staining vessel for extended periods of time. This stabilizes the stain for a longer time in storage and helps to control the rate of nuclear staining.[4] Staining is believed to occur due to positively charged aluminum-hematein chelates combining with the negatively charged phosphoric acid groups of DNA.

DIRECTIONS FOR USE

Use the Gill's Hematoxylin formulations at full strength. No mixing, dilution, or further treatment is required. An alternative to diliuting formulas No. 2 and No. 3 is to use Gill's No. 1 with longer staining times. Filtration before use for the first time is not needed. Prior to repeated use, filtration is recommended for cytology to control cellular cross-contamination.[8]

CYTOLOGY SPECIMEN PREPARATION

Best results are obtained with fresh, unfixed material that is spread on a slide, or collected on a membrane filter, and wet-fixed immediately in 95% ethyl alcohol. If ethyl alcohol is not available, one of the following alternatives is recommended: (1) absolute methyl alcohol (not for Millipore filters),[9-10] (2) 95% reagent grade alcohol,[9-10] (3) 80% isopropyl alcohol,[9] or (4) 90% acetone[11] (not for Millipore filters). Fixed air-dried cells coated with water-soluble wax, used in cytology for the protection and transport of the specimen, should be immersed in 95% ethyl alcohol for at least 10 minutes before staining to complete fixation and to remove the wax. Wax not removed slows or stops the rate of staining and unless the staining time is increased, will result in hypochromatic chromatin. Except for cells protected by water-soluble wax, wet-fixed cells must always be kept wet. Air-drying before or after fixation must not occur.[10]

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HISTOLOGY SECTIONS

Tissue fixed by any type of fixative is suitable. Fixatives that contain mercuric chloride produce mercury deposits that must be removed from the sections before staining. The necessary details are given in standard histological texts, for example Lugol's Iodine can be used, often with a Sodium Thiosulfate reducer series to remove the mercury deposits formed during fixation.[12-13] If the deposits are not removed they will appear as fine black granular precipitate on the tissue sections.

RESULTS

Chromatin is stained blue to blue-black. Nucleoli are delicately stained and display eosinophilia, assuming subsequent staining in Eosin Y as the counterstain. Barr bodies are conspicuously stained. Cytoplasm is scarcely tinted so that differentiation in acid is omitted. Staining 2500 slides per week, 4 liters of Gill's Hematoxylin No. 1 will last approximately 2 to 4 months.

STORAGE

Follow general good practice for storage of dye solutions. Keep stock solutions in the dark at room temperature. Store in tightly capped containers. To minimize air oxidation of hematoxylin and thereby extend the shelf life of the solution allow little or no air-space.[14] Shelf life is normally 2 years. Continued usefulness of hematoxylin solution is best tested microscopically. Stain a test slide (e.g., a buccal smear for cytology or deparaffinized tissue or frozen section) and compare with usual results.

INDICATIONS OF DETERIORATION DURING STORAGE OR USE

Stock solutions of Gill's Hematoxylin that become brown either contain too much acetic acid or have become over-oxidized. Over acidified hematoxylin stains very slowly, if at all, while over-oxidized hematoxylin stains chromatin an unattractive brownish color. In-use solutions of Gill's Hematoxylin that become purple have had their acetic acid diminished or exhausted by evaporation. Such solutions begin to lose their selectivity for chromatin and begin staining cytoplasm as well. Also, an unused solution becomes more concentrated as some of its intentionally un-oxidized hematoxylin is converted to hematein by contact with atmospheric oxygen at the solution-air interface. A metallic sheen or scum may form if solutions of Gill's Hematoxylin No. 3 are overexposed to air or left undisturbed for a long period of time.

WARNING

HARMFUL IF SWALLOWED. DO NOT DRINK. Contains Ethylene glycol (approximately 25% v/v) and aluminum sulfate.

ORDERING INFORMATION

Cat.#	Description	Size
24242	Gill's Hematoxylin No. 1 for Cytology	500mL 1000mL
24243	Gill's Hematoxylin No. 2 for Histology and Cytology (double strength)	500mL 1000mL
24244	Gill's Hematoxylin No. 3 for Histology (triple strength)	500mL 1000mL
09782	Gill's Modified OG-6	500mL 1000mL 3.75L
09783	Gill's Modified EA	500mL 1000mL 3.75L
09859	Eosin Y, 0.5% Alcoholic (contains acetic acid)	500mL 1000mL 3.75L
17269	Eosin Y, 1% Alcoholic (contains no acetic acid)	500mL 1000mL

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