

Thermo Scientific™ Richard-Allan Scientific™ Chromaview™ – Advanced Testing Methyl Green-Pyronin Y Stain Solution Instructions for Use

For in vitro diagnostic use.

For use as a kit in special stain techniques.

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

Frozen sections fixed in methanol is the procedure and fixative of choice; Carnoy's Fixative can be used for paraffin sections. Formalin fixed tissue is adequate for demonstration of plasma cells.

Quality Control

A section containing many plasma cells should be used.

Technical Procedure

Working Methyl Green-Pyronin Y Solution

Methyl Green-Pyronin Y Stock Solution.....5 mL
Deionized Water.....50 mL

Frozen Section Staining Protocol

1. Fix sections in methyl alcohol for 5 minutes.
2. Stain sections in Methyl Green-Pyronin Y Working Solution for 5 minutes at room temperature.
3. Rinse sections thoroughly in deionized water.
4. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
5. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Paraffin Section Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Rinse sections in deionized water for 1 minute.
3. Stain sections in Methyl Green-Pyronin Y Working Solution for 5 minutes at room temperature.
4. Rinse sections thoroughly in deionized water.
5. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
6. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Deoxyribonucleic Acid (DNA) – Green to Aqua
Ribonucleic Acid (RNA) – Red

Discussion

The Methyl Green-Pyronin Y Stain Solution should be stored at room temperature. If contamination occurs, discard the solution. The Methyl Green-Pyronin Y Stain Solution is for "In Vitro" use only. Refer to the Safety Data Sheet for Health and Safety Information. This solution is stable and should not form precipitants under ordinary storage parameters. It is recommended that the Methyl Green-Pyronin Y Working Solution be discarded after use. All dyes used in this formulation are certified by the Biological Stain Commission.

Technical Comments

When this stain is used undiluted, the Pyronin Y hue is enhanced and the green to red contrast ratio is reduced. The green hue may turn more red-violet. When frozen sections are fixed in methanol, DNA always appears green and RNA appears red (present in both the nucleolus and the cytoplasm). With Carnoy's fixed tissue, results are similar to frozen section/methanol fixed procedures. Blood films or bone marrow smears should be air dried, fixed in methanol, air dried again and stained using the frozen section procedure. Formalin fixed tissue may be used to demonstrate plasma cells but is not the fixation method of choice.

Probable Mode of Action

There are two types of nucleic acids found in the nuclear chromatin of cells: DNA (Deoxyribonucleic acid) found in the nucleus of cells, and RNA (Ribonucleic acid) found in the nucleolus. RNA is also present in the cytoplasm of cells. The technique most widely used to distinguish DNA and RNA in tissue sections is the Methyl Green-Pyronin Y method.

The probable mechanism of this reaction is based upon the competition between the slow staining, but doubly charged, methyl green and the more rapidly staining, singly charged pyronin Y. Methyl green has two cationic charged groups that become linked to the phosphate moieties in the DNA. The pyronin Y displaces the methyl green from all sites of linkage except where its double charge gives it a selective advantage (acidic polymer such as DNA). Consequently the methyl green stains DNA, and retains its binding to this substance against the competitive action of pyronin Y. Pyronin Y stains the less polymerized RNA rapidly and it can displace methyl green from linkages having smaller polymeric acidic substances (RNA).

The reaction is done at pH 4.2 – pH 4.3. The advantage of a low pH is to have the nucleic acids in a charged condition and in their least soluble state.

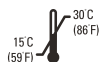
References

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Order Information

Product	Size	Qty.	REF
Methyl Green-Pyronin Y (MGP)	125 mL	1	87011

Anatomical Pathology



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