

Thermo Scientific Rapid-Chrome Iron Stain and Thermo Scientific Rapid-Chrome Nuclear Fast Red Counterstain Instructions for Use

**For in vitro diagnostic use.
For use as a special stain in laboratory applications.**

PLEASE READ THIS PACKAGE INSERT IN ITS ENTIRETY BEFORE USING THE PRODUCT.

Intended Use

For demonstration of hemosiderin or non-hemoglobin iron in cells and tissue by the Prussian blue staining method.

Contents

Each iron stain dropper contains one crushable glass ampoule (0.6 mL) of each:

- 2% Hydrochloric Acid
- 2% Potassium Ferrocyanide

Each dropper of fast red counterstain contains one crushable glass ampoule (1.2 mL) of 0.1% Nuclear Fast Red in aqueous %5 aluminum sulfate, with 0.01% Thymol as a preservative.

Each dropper is sufficient to stain approximately 4 slides, depending on size of sample, and comes fitted with a filtering dropper tip.

Introduction

The Prussian Blue staining reaction allows for visualization of free or loosely-bound iron in cells and tissue. Examples include siderocytes (red blood cells containing non-hemoglobin iron-containing granules), hemosiderosis (deposits of crystalline ferritin aggregates), and hemochromatosis (iron deposits in liver and pancreas). Spleen and bone marrow also normally contain small quantities of ferric iron.

Loosely bound iron is easily released by treatment with mild acid (2% hydrochloric acid). Free ferric ions then combine with the ferrocyanide to form the bright blue or blue-green Prussian Blue pigment (ferric ferrocyanide). Visualization is aided by counterstaining cell nuclei with nuclear fast red.

Instructions for Use

The Thermo Scientific™ Shandon™ Rapid-Chrome Iron Stain and Thermo Scientific™ Rapid-Chrome Nuclear Fast Red Counterstain allow this procedure to be performed quickly and easily without premixing or handling any dangerous chemicals. The convenient dropper tips provide freshly mixed and filtered staining solution at a moment's notice.

Results

Rapid-Chrome Iron Stain

1. Prepare the slides:
 - a. For paraffin-embedded sections, deparaffinize and hydrate to distilled water. For best results, the pH of your water should not be greater than 7.0.
 - b. For blood or bone marrow smears, air-dry, fix in absolute methanol for 15 minutes at room temperature, and air-dry.

Note: A positive control slide of the same tissue type should be included in each staining run.

2. Place the slides in a humidity chamber to prevent evaporation of the staining solution.
3. Crush each of the two ampoules in the iron stain dropper by pressing at the dots on the label. DO NOT BEND the plastic dropper.
4. Mix the two solutions in the dropper by inverting the dropper several times. DO NOT SQUEEZE the dropper until you are ready to dispense the solution.
5. IMMEDIATELY dispense the solution onto the slides by squeezing the dropper unit. Be sure to completely cover the specimen with staining solution. Discard the used dropper.
6. Incubate the slides in the humidity chamber at room temperature for 30 minutes or until sufficient stain intensity is achieved. The solution can be left on the sample of up to an hour. Incubating at a higher temperature, up to 60° C., will speed up the reaction. At elevated temperatures, limit the reaction time to 30 minutes.
7. Rinse the slide thoroughly with distilled water.

Rapid-Chrome Nuclear Fast Red Counterstain

8. If desired, counterstain the slides with Nuclear Fast Red by crushing the single glass ampoule in the counterstain dropper and squeezing the stain onto the slide.

9. Incubate at room temperature for at least 1 minute or until desired color intensity is achieved.

10. Rinse thoroughly with distilled water.

11. Dehydrate, clear and coverslip using a synthetic mounting medium, such as Thermo Scientific™ Shandon-Mount™, Thermo Scientific™ EZ-Mount™, or Thermo Scientific™ Consul-Mount™.

Precautions

Iron deposits or hemosiderin will appear bright blue to bright blue-green. When counterstained with Nuclear Fast Red, nuclei will appear red and cytoplasm will appear light pink.

Treatment of cells with hydrogen peroxide prior to performing the staining reaction may cause false positive results due to release of hemoglobin iron from red blood cells.

Use only distilled water in all solutions to which the specimen is exposed. Ions in tap water may cause some false positive or background staining.

Do not break the ampoules in the iron stain dropper until you are ready to dispense the reagents onto the slides, as the solution must be used fresh. Mix the solution thoroughly by inverting the dropper several times before dispensing.

Storage

Store at room temperature.

References

Clark, G., ed. Staining Procedures, 4th ed., Williams & Wilkins, Baltimore. 1981;
Sheehan, D. C. and Hrapchak, B. B., ed. Theory and Practice of Histotechnology, 2nd ed., Battelle Press, Columbus. 1980

Warning and Precautions

FOR IN VITRO DIAGNOSTIC USE

**See Safety Data Sheets for warnings and precautions, as well as EUH code definitions.
See container label for warnings and precautions.**

Order Information

Product	Qty.	REF
Rapid-Chrome Iron Stain Sticks	25 Droppers	9990120
Rapid-Chrome Nuclear Fast Red Counterstain Sticks	25 Droppers	9990121
Rapid-Chrome Iron Stain and Nuclear Fast Red Counterstain Kit	50 Droppers (25 of each)	9990122

