

**INTENDED USE**

The Iron Stain Kit employs the Prussian blue (ferric ferrocyanide) reaction to demonstrate iron deposits in tissue sections. Brazilliant! is used as a red nuclear stain.

**PRODUCT SUMMARY**

Hemosiderin is demonstrated in tissues with the Perl's Prussian blue method. The iron (ferric ion) component of the hemosiderin cannot chemically react because it is complexed to a protein. Exposure of hemosiderin to a solution of hydrochloric acid releases the ferric ion from the protein. The ferric ion then reacts with potassium ferrocyanide to form the Prussian blue precipitate, ferric ferrocyanide. Iron deposits stain blue.

Brazilliant! is formulated like Harris Hematoxylin, substituting the natural dye Brazilin for Hematoxylin. Brazilin is oxidized to brazilein with sodium iodate, then complexed with aluminum.

Since Hematoxylin and Brazilin dyes differ only by one hydroxyl group, the mechanism of dye to tissue staining is the same. In all likelihood, the aluminum atom forms coordinate bonds to nucleic acids. Additionally, four hydroxyls on the alum-brazilein complex offer sites for hydrogen bonding. At suitably low pH, staining is limited to DNA and RNA.

**INGREDIENTS**

2% potassium ferrocyanide

4% hydrochloric acid

Brazilliant!: acetic acid, aluminum ammonium sulfate, reagent alcohol, Brazilin, sodium iodate

**WARNING**

2% potassium ferrocyanide: Irritant. Avoid contact with skin and eyes.

4% hydrochloric acid: Corrosive. Irritant. Avoid contact with skin and eyes.

Brazilliant!: Irritant. Avoid contact with skin and eyes.

For In Vitro Diagnostic Use.

**STORAGE**

Store kit at room temperature, away from direct sunlight. Keep all containers tightly closed when not in use.

**DIRECTIONS FOR USE**

1. Due to the acid nature of decal solutions, specimens that have been decaled (e.g., bone marrow core biopsies) may lose iron deposits resulting in loss of stainable iron.
2. Staining time of Brazilliant! is dependent upon section thickness and desired color intensity.

**RECOMMENDED STAINING SCHEDULE**

1. Clearant x 3 .....3 minutes each
2. 100% alcohol x 2 .....1 minute each
3. 95% alcohol .....1 minute
4. 50% alcohol .....1 minute
5. Distilled or deionized water x 2 .....30 seconds each
6. Working Iron solution
  - a. Just before use, mix together equal parts:
    - i. 2% potassium ferrocyanide
    - ii. 4% hydrochloric acid
  - b. Immerse slide .....30 minutes
7. Tap water x 2 .....30 seconds each
8. Brazilliant! Filter before use.....5-10 minutes
9. Distilled or deionized water x 2 .....30 seconds each
10. 70% alcohol .....1 minute
11. 95% alcohol .....1 minute
12. 100% alcohol x 3 .....1 minute each
13. Clearant x 3 .....1 minute each

Results: Hemosiderin (iron)—bright blue; Nuclei—red

**DISPOSAL**

1. Use a licensed waste hauler.
2. Discard into the sanitary sewer system with the approval of local wastewater officials.

**MSDS**

MSDS are available online at [www.anatechltdusa.com](http://www.anatechltdusa.com).

**ORDERING INFORMATION FOR IRON KIT**

<u>Cat#</u>	<u>Packaging</u>
883	Iron Stain Kit