

**INTENDED USE**

Wright Stain is intended for use in staining blood films or bone marrow films. Solutions are for "In Vitro Diagnostic Use."

Wright Stain is a modified Romanowsky stain intended for differentially staining the cellular elements of blood. When blood films are treated as herein described, the white blood cell nucleus and cytoplasm take on characteristic blue or pink coloration. The purified dyes in the ACCUSTAIN formulations of Wright Stain eliminate inconsistent staining and yield reproducible lot-to-lot chromogenic responses.

The procedures in this insert describe the use of Wright Stain as a manual dip stain or for use in batch stainers such as the Hemastainer supplied by Geometric Data, the Midas II supplied by EM Diagnostic Systems, Inc. and the Fisher Stainmaster supplied by Fisher Scientific.

**REAGENTS**

**WRIGHT STAIN, MODIFIED, Catalog No. WS**

Wright Stain, modified 0.3% w/v, buffered at pH 6.8 in methanol.

**PHOSPHATE BUFFER, Catalog No. P 3288**

A mixture of sodium phosphate and potassium phosphate, 0.0083 M/L, pH 7.2.

**RINSE SOLUTION 2, Catalog No. RS 2**

Ethanol solution, 18%, of wetting agent. Contains 0.02% sodium azide as preservative.

**METHANOL, ACETONE FREE, Catalog No. M 1775**

**STORAGE AND STABILITY:**

Store Wright solutions at room temperature (18-26°C). Reagent label bears expiration date.

Store Phosphate Buffer, Rinse Solution 2 and Methanol at room temperature (18-26°C).

Store working phosphate buffer solution at 2-8°C. Warm before use.

**DETERIORATION:**

Discard Wright stain solutions if a precipitate develops. Discard the working phosphate buffer solution if turbidity or visible bacterial growth is present.

**PREPARATION:**

Wright Stain solution is supplied ready to use, although Wright stain may be diluted if placed on an automated instrument. See instructions which follow.

The phosphate buffer (P3288) should be prepared by diluting 1 vial of buffer to 1 gallon or 3.8 liters of deionized water. Mix well to dissolve.

Methanol is ready to use.

**PRECAUTIONS:**

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

**US Risk and Safety Statements**

Wright Stain is **FLAMMABLE** and **TOXIC**. Toxic by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition – no smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Rinse Solution 2 is **COMBUSTIBLE** and **HARMFUL**. Flammable. Harmful if swallowed. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition – no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves. Sodium azide may react with lead and copper to form highly explosive compounds. Target organs: Nerves and liver.

Phosphate buffer. Caution: Substance not yet fully tested.

Methanol is **FLAMMABLE** and **TOXIC**. Toxic by inhalation, in contact with skin and if swallowed. Toxic: Danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition – no smoking. Take precautionary measures against static discharges. Avoid contact with skin. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**EU Risk and Safety Statements (Caution: Substances not yet fully tested)**

Wright Stain and Methanol are **HIGHLY FLAMMABLE** and **TOXIC**. Highly flammable. Toxic by inhalation, in contact with skin and if swallowed. Toxic: Danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Keep container tightly closed. Keep away from sources of ignition – no smoking. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing and gloves.

Rinse Solution 2 is an **IRRITANT**. Flammable. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition – no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Phosphate buffer. Caution: Substance not yet fully tested.

**SPECIMEN COLLECTION:**

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Fresh whole blood films or fresh films from blood anticoagulated with EDTA must be used. Prior to preparation of films, blood must be thoroughly mixed at room temperature (18-26 °C). The films should be prepared within 1 hour of blood collection. If not stained the same day, slides should be fixed in absolute methanol and stored in a dust-free container.

**SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:**

Microscope

Slides

Coverslips

**NOTES:**

1. For greater cellular detail, staining time may be increased. Color (tones of blue or red) may be varied by increasing or decreasing time in deionized water.
2. Rapid (15 seconds) staining is not recommended for bone marrow. For those preparations 1-3 minutes in stain and 2-6 minutes in deionized water gives satisfactory results.
3. For batch staining, slide racks and dishes such as supplied by Miles Scientific for Tissue-Tek® are recommended since this system allows for vertical placement of slides.
4. Color can be varied by increasing or decreasing time in deionized water. Bone marrows should be stained for at least 90 seconds and buffered for 90 seconds to 3 minutes.
5. Staining times outlined in the above procedures have given satisfactory results in our laboratories. Individual preferences may dictate adjustment of these times.
6. The manual procedure times may also be used with the Hemastainer provided swing is in the off position.
7. Timing may be varied to suit individual preference. (Automated procedures)
8. Water rinses should be deionized water. (Automated procedures)
9. If deionized water is not neutral pH, we suggest the use of Phosphate Buffer, pH 7.2, Catalog No. P 3288.
10. Positive control slides should be included in each run.
11. The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

**PROCEDURE:**

**I. Dip Method (Rapid – Manual)**

1. Place approximately 50 ml WRIGHT STAIN in a Coplin jar.  
NOTE: KEEP TIGHTLY SEALED WHEN NOT IN USE. Replace when water artifacts appear in red cells or when a precipitate is evident.
2. Fill another Coplin jar with deionized water.
3. Place thoroughly dried blood film, feather edge DOWN, in WRIGHT STAIN for approximately 15 seconds.  
NOTE: Rapid dipping for 5-10 seconds may reduce water artifacts on films that are not thoroughly dried.
4. Remove slides from stain and place in deionized water, feather edge DOWN, for approximately 30 seconds. DO NOT AGITATE SLIDE WHILE IT IS IN DEIONIZED WATER.
5. Rinse briefly in running deionized water and air dry thoroughly before evaluation.

**II. Horizontal Staining Method (Manual)**

1. Place thoroughly dried blood film on an appropriate staining rack.
2. Flood slide with 1-2 ml WRIGHT STAIN.
3. After 30 seconds, without rinsing off Wright Stain from Step 2, add an equal volume of deionized water and mix thoroughly by gently blowing on slide.
4. After 1 minute, thoroughly rinse with deionized water and air dry.

**Batch Staining With Hemastainer**

1. Set timers to provide the following times for each station:  
Station 1 - 30 seconds  
Station 2 - 2 minutes  
Station 3 - 3.5 minutes  
Station 4 - 30 seconds  
Station 5 - Skip and go to "air dry"
2. Prepare stations for staining in the following manner:  
Station 1 - Absolute Methanol, 500 ml  
Station 2 - ACCUSTAIN Wright Stain, 350 ml and 150 ml of Absolute Methanol  
Station 3 - 500 ml Phosphate Buffer, pH 7.2  
Station 4 - 3.8 L of deionized water and 100 ml Phosphate Buffer  
Station 5 - Leave empty
3. Turn power switch to ON.
4. Set Auto-Manual switch to MANUAL.
5. Set Right/Left switch to LEFT.
6. Set swing switch to ON.
7. Set pump switch to AUTO.
8. Load basket with thoroughly air-dried blood films.
9. Attach basket to hanger and tighten.
10. Start process by setting Auto/Manual switch to AUTO.
11. When cycle is complete and slides are thoroughly dry, set Auto/Manual switch to MANUAL. The basket will then return to starting position.

**Batch Staining With Fisher Stainmaster**

Set program as follows:

Event	Station	Reagent	Time (Minutes)
1	1	Absolute Methanol	0.5
2	2	Wright Stain, Modified, Catalog No. WS-128	1.5
3	3	Phosphate Buffer, Catalog No. P 3288	1.0
4	6	Deionized water	0.3
5	5	Rinse Solution 2, Catalog No. RS 2	0.7
6	4	Deionized water	0.3
7	Dry	Air	5.0

**Batch Staining With Midas II**

Set program as follows:

Step	Bath	Reagent	Time (Seconds)
1	1	Absolute Methanol	30
2	2	Wright Stain, Modified, Catalog No. WS-128	60-90
3	3	Phosphate Buffer, Catalog No. P 3288	60
4	4	Running deionized water	10
5	Dry	Air	3 minutes or until dry

Unused baths may be omitted.

**PERFORMANCE CHARACTERISTICS**

Nuclei will be varying shades of purple. Cytoplasmic staining will be varying shades of blue to light pink. Fine reddish to lilac granules may be present in cytoplasm of some cell types. Basophils will demonstrate dark blue black granules in the cytoplasm. Eosinophils will demonstrate bright orange granules in the cytoplasm. Red blood cells should be pink to orange.<sup>1</sup>



If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

**REFERENCES**

1. Hematology: Principles and Procedures, Sixth Edition, Brown AB, Lea & Febiger, Philadelphia 1993 p101

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