



**ACCUSTAIN®  
WRIGHT STAIN**  
(Procedure No. WSGD-128)

**SIGMA-ALDRICH®**

For Geometric Data Hemastainer® and Hematrak®

**INTENDED USE**

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

Wright Stain, Catalog No. WSGD-128, is a modified Romanowsky stain used 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. Wright Stain, Catalog No. WSGD-128, is used in batch stainers such as the Hemastainer® supplied by Geometric Data.

**REAGENT**

**WRIGHT STAIN**, Catalog No. WSGD-128  
Wright Stain, 0.14% w/v, with stabilizers in methanol.

**SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:**

**PHOSPHATE BUFFER**, Catalog No. P3288

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

**PHOSPHATE BUFFER**, Catalog No. P8165

A mixture of sodium phosphate and potassium phosphate, 0.034 M/L, pH 6.6

**METHANOL, Absolute, Acetone Free**, Catalog No. M1775

Microscope / Slides / Coverslips / Geometric Data Hemastainer® and accessories / Geometric Data Hematrak® and accessories

**STORAGE AND STABILITY:**

Store stock Wright Stain solution at room temperature (18-26°C). Keep container tightly closed. Reagent label bears expiration date. Replace working Wright Stain solution for Station 3 if more than 250 slides per day are stained, or if the platelets stain lightly. If continued light staining is observed, solution at Station 2 should also be changed. The solutions at all stations should be changed daily.

Store Phosphate Buffers and Methanol at room temperature (18-26°C). Store working phosphate buffer solution at 2-8°C.

**DETERIORATION:**

Discard Wright Stain or Phosphate Buffer if turbidity develops.

Discard Wright stain solution if a precipitate develops or water artifacts appear in red cells. Discard the working phosphate buffer solution if turbidity or visible bacterial growth is present.

**PREPARATION:**

Phosphate Buffer Solutions are prepared by dissolving contents of one vial of Phosphate Buffer in 3.8 liters deionized water.

NOTE: Variation in pH of water may have a significant effect on the performance of this reagent on the Hemastainer®. It is recommended that the final pH of the buffer be confirmed to ensure optimum performance.

Station 1 Reagent is Methanol, Catalog No. M 1775.

Station 2 Reagent is Wright Stain/Methanol Solution and is prepared by mixing 350 ml Wright Stain with 150 ml Methanol, Catalog No. M 1775.

Station 3 Reagent is a Wright Stain/Phosphate Buffer Solution and is prepared by mixing 100 ml Wright Stain with 400 ml of Phosphate Buffer Solution.

Station 4 Reagent is buffered deionized water and is prepared by mixing 3.8 liters deionized water with 200 ml Phosphate Buffer Solution.

Station 5 Reagent is Phosphate Buffer Solution.

**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 solution is FLAMMABLE and TOXIC. Toxic by inhalation, in contact with skin and if swallowed. May cause cancer. May cause heritable genetic damage. Irritating to eyes, respiratory system and skin. 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). Avoid exposure – obtain special instructions before use. Avoid contact with skin.

Phosphate buffers. 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 solution and Methanol are HIGHLY FLAMMABLE and TOXIC. Highly flammable. Toxic by inhalation, in contact with skin and if swallowed. May cause cancer. May cause heritable genetic damage. Irritating to eyes, respiratory system and skin. 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). Avoid exposure – obtain special instructions before use. Avoid contact with skin.

Phosphate buffer. Caution: Substance not yet fully tested.

Methanol is 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. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**PROCEDURE**

**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. If the stained sample is to be evaluated with the Hematrak® system, a long wedge film with a straight, even feathered edge is required. Geometric Data Miniprep Automatic Smearing Device will provide an adequate specimen. Prior to preparation of films, blood must be thoroughly mixed at room temperature (18-26°C).

**NOTES:**

1. If the Geometric Data Hematrak® is used, please see the owner's manual for instructions.
2. Use Phosphate Buffer, pH 6.6 if the slides are intended to be read by the Geometric Data Hematrak®. If the slides are intended to be read on a regular light microscope, use Phosphate Buffer, pH 7.2.
3. Cover Station 1 and Station 2 when not in use.
4. For proper performance, leave Station 3 uncovered.
5. This staining program has provided satisfactory results at Sigma-Aldrich. Personal preference and environmental conditions may dictate adjustment of staining times.
6. In case the slides cannot be immediately stained, the slides should be fixed in methanol for 1 minute and dried. Stain as soon as possible.
7. Positive control slides should be included in each run.
8. 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:**

**PROCEDURE FOR BATCH STAINING  
WITH HEMASTAINER®**

Station	Reagent	Time
1	Methanol	15-30 seconds
2	Wright Stain/Methanol	2.0 minutes
3	Wright Stain/Phosphate Buffer	5.0 minutes
4	Deionized Water/Phosphate Buffer	20 seconds
5	Phosphate Buffer	1.0 minutes

**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 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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