

INTENDED USE

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

Wright-Giemsa Stain is a modified Romanowsky stain used for differentially staining the cellular elements of blood. When blood films are stained as described in the procedure, the white blood cell nucleus and cytoplasm take on the characteristic blue or pink coloration. The combination of purified eosin and thiazine dyes in the product eliminates inconsistent staining and yields lot-to-lot reproducible chromogenic response.

REAGENT

WRIGHT-GIEMSA STAIN, MODIFIED

Wright-Giemsa stain, modified, 0.4% w/v, buffered at pH 6.8, in methanol.

PHOSPHATE BUFFER, Catalog No. P3288

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

METHANOL, Acetone Free, Catalog No. M1775

STORAGE AND STABILITY:

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

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

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

PREPARATION:

Wright-Giemsa solution is supplied ready to use.

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-Giemsa solution is **FLAMMABLE** and **TOXIC**. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation 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 discharge. 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).

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 discharge. 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-Giemsa solution 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.

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 should be used. Thicker films (e.g., bone marrow) will usually require longer staining times.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Microscope
Slides
Coverslips

NOTES:

- 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.
- Rapid (30 seconds) staining is not recommended for bone marrow. For those preparations 1-3 minutes in stain and 2-10 minutes in deionized water gives satisfactory results.
- 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.
- Color can be varied by increasing or decreasing time in deionized water. Bone marrows should be stained for at least 3 minutes and buffered for 3-10 minutes.
- Staining times outlined in the above procedures have given satisfactory results in our laboratories. Individual preferences may dictate adjustment of these times.
- The deionized water recommended for this procedure should be between pH 6.8 to 7.2. If the deionized water is more acidic than pH 6.8, a phosphate buffer is recommended.
- In case the slides cannot be immediately stained, the slides can be fixed in methanol for 1 minute and dried. Stain as soon as possible.
- Positive control slides should be included in each run.
- 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)

- Place approximately 50 ml Wright-Giemsa Stain in a Coplin jar.
- Fill another Coplin jar with water or phosphate buffer.
- Place thoroughly dried blood film, feather edge DOWN, in Wright-Giemsa Stain for approximately 30 seconds. NOTE: Rapid dipping for 5-10 seconds may reduce water artifacts on films that are not thoroughly dried.
- Remove slide from stain and place in deionized water or phosphate buffer, pH 6.8-7.2, feather edge DOWN, for approximately 1-10 minutes. DO NOT AGITATE SLIDE WHILE IT IS IN DEIONIZED WATER.
- Rinse briefly in running deionized water and air dry thoroughly before evaluation.

II. Horizontal Staining Method

- Place thoroughly dried blood film on an appropriate staining rack.
- Flood slide with 1-2 ml Wright-Giemsa Stain.
- After 1 minute, add an equal volume of deionized water or phosphate buffer, pH 6.8-7.2, and mix thoroughly by gently blowing on slide.
- After 1-3 minutes, thoroughly rinse with deionized water and air dry.

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.¹



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

REFERENCES

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

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