

## INTENDED USE

Sigma-Aldrich Acid Phosphatase kit is intended for use in the cytologic demonstration of acid phosphatase activity in leukocytes from blood, bone marrow and tissue touch preparations. Acid Phosphatase reagents are for "In Vitro Diagnostic Use."

Use of substituted naphthol AS phosphates in conjunction with diazonium salts for detection of acid phosphatase in human leukocytes was first reported by Goldberg and Barka.<sup>1</sup> The Sigma-Aldrich procedure for demonstrating this leukocyte enzyme is based upon use of naphthol AS-BI phosphate and fast garnet GBC salt. The latter diazo compound was selected because it couples rapidly at acid pH, forming highly insoluble dye deposits. Tartaric acid is provided in order to demonstrate the presence of tartrate-resistant acid phosphatase.

According to the Sigma-Aldrich procedure, blood films are incubated in a solution containing naphthol AS-BI phosphoric acid and fast garnet GBC. Duplicate films are treated with L(+)-tartrate-containing substrate. Naphthol AS-BI, released by enzymatic hydrolysis, couples immediately with fast garnet GBC forming insoluble maroon dye deposits at sites of activity. Cells containing tartaric acid-sensitive acid phosphatase are devoid of activity, whereas those mononuclear cells containing tartaric acid-resistant phosphatase are not affected by such treatment.

## REAGENTS

### CITRATE CONCENTRATE, Catalog No. 386-1

Citrate buffer, 0.38 mol/l, pH 5.4 when diluted according to procedure.

### TARTRATE SOLUTION, Catalog No. 386-2

L(+)-Tartrate buffer, 0.67 mol/l, pH 5.2 when diluted according to procedure.

### ACETATE SOLUTION, Catalog No. 386-3

Acetate buffer, 2.5 mol/l, pH 5.2 when diluted according to procedure.

### NAPHTHOL AS-BI PHOSPHORIC ACID SOLUTION, Catalog No. 386-4

Naphthol AS-BI phosphoric acid, 12.5 mg/ml, in N,N'-dimethyl formamide.

### FAST GARNET GBC SALT, Catalog No. 386-15

Fast garnet GBC salt, purified, 15 mg/capsule.

### ACID HEMATOXYLIN SOLUTION, Catalog No. 285-2

Hematoxylin, certified, 1 g/l, sodium iodate, aluminum ammonium sulfate, and stabilizers, pH 3.3 at 25°C.

### STORAGE AND STABILITY

Store Citrate Concentrate, Tartrate Solution and Acetate Solution in refrigerator (2–8°C). Discard if turbidity develops.

Store Fast Garnet GBC Salt and Naphthol AS-BI Phosphoric Acid Solution in refrigerator (2–8°C). Color of Naphthol AS-BI Phosphoric Acid Solution may vary from pale to dark yellow. Reagents are stable until expiration date shown on bottles.

Store Acid Hematoxylin Solution at room temperature (18–26°C). Discard when time for suitable staining exceeds time recommended in procedure by more than 5 minutes.

Dilute citrate solution is suitable for use in the absence of visible microbial growth. Citrate/Acetone solution should be kept tightly capped when not in use. Prepare fresh daily.

### PREPARATION:

Dilute Citrate solution is prepared by adding 2.0 ml Citrate Concentrate, Catalog No. 386-1 to 18.0 ml water.

The Citrate/Acetone fixative is prepared by adding 20.0 ml dilute citrate solution to 30.0 ml acetone.

NOTE: Keep Citrate/Acetone Solution tightly capped when not in use. Prepare fresh daily.

### 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 Risks and Safety Statements

Citrate Solution is CORROSIVE. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of an accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Tartrate Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Acetate Solution. Caution: Substance not yet fully tested.

Naphthol AS-BI Phosphoric Acid Solution is TOXIC. Harmful by inhalation and in contact with skin. Irritating to eyes, respiratory system and skin. May cause harm to the unborn child. Do not breathe vapor. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible). Avoid exposure - obtain special instructions before use.

Fast Garnet GBC Salt is TOXIC. May cause cancer. Do not breathe dust. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible). Avoid exposure - obtain special instructions before use.

Acid Hematoxylin is TOXIC. Toxic if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Acetone is FLAMMABLE and an IRRITANT. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Target organs: Liver and kidneys.

### EU Risks and Safety Statements

Citrate Solution is an IRRITANT. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Tartrate Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Acetate Solution. Caution: Substance not yet fully tested.

Naphthol AS-BI Phosphoric Acid Solution is TOXIC. Harmful by inhalation and in contact with skin. Irritating to eyes, respiratory system and skin. May cause harm to the unborn child. Do not breathe vapor. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible). Avoid exposure - obtain special instructions before use.

Fast Garnet GBC Salt is TOXIC. May cause cancer. Do not breathe dust. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible). Avoid exposure - obtain special instructions before use.

Acid Hematoxylin is HARMFUL. Harmful if swallowed.

Acetone is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

## 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.

Blood, bone marrow films, tissue touch preparations and cytocentrifuge preparations may be used. Either EDTA or heparin will serve as anticoagulants. Blood or bone marrow films may be stored fixed at room temperature (18–26°C) for several weeks or unfixed for several days without appreciable change in activity.<sup>2</sup> Do not ship whole blood for assay at other laboratories. Send fixed or unfixed slides. Slides should be kept cool during transit. Allow films to dry at least 1 hour prior to fixation.

### SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Acetone, ACS Reagent  
37°C water bath

### NOTES:

Test performance can be monitored by including blood films prepared from a healthy individual. In the absence of tartaric acid, all leukocytes demonstrate granular sites of activity. When incubated with tartrate, an occasional granule may be observed in lymphocytes, but all other normally occurring cells are devoid of activity.

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:

#### Fixation:

1. Fix slide in Citrate/Acetone Solution for 30 seconds at room temperature (18–26°C).
2. Rinse slides in deionized water and allow to air dry for at least 15 minutes.

#### Staining:

1. Label two 100-ml beakers A and B and add the following, mixing after each addition:

	Beaker A	Beaker B
Water, prewarmed to 37°C	46.0 ml	44.0 ml
Acetate Solution, Catalog No. 386-3	2.0 ml	2.0 ml
Naphthol AS-BI Phosphoric Acid, Catalog No. 386-4	2.0 ml	2.0 ml
Tartrate Solution, Catalog No. 386-2	—	2.0 ml

2. Add contents of 1 capsule Fast Garnet GBC Salt, Catalog No. 386-15, to each beaker. Stir on magnetic mixer 30–60 seconds.
3. Rapidly filter solutions through Whatman No. 54 (or equivalent) paper into separate labeled staining jars.
4. Warm solutions in staining jars to 37°C in water bath. Check that temperature is at 37°C before adding slides.
5. Place at least 2 duplicate fixed blood films in each jar containing the filtered substrate solution.
6. Incubate slides 1 hour at 37°C in the dark.

7. Remove slides and wash in deionized water for 3 minutes.
8. Stain in Acid Hematoxylin Solution, Catalog No. 285-2, for 5 minutes.
9. Rinse slides in deionized water for 3 minutes and allow to air dry.
10. Evaluate microscopically using oil immersion (900X) lens. Use of solvent based mounting media is not recommended as they may cause fading of the stains.

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## PERFORMANCE CHARACTERISTICS

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Stained blood films are usually evaluated subjectively for the presence or absence of tartrate-resistant enzyme. In the absence of tartaric acid, most leukocytes demonstrate granular sites of activity. When incubated with tartrate, an occasional granule may be observed in lymphocytes and some specialized macrophages, such as Gaucher's cell, epithelial cells and hairy cells.<sup>3</sup> In blood smears a positive reaction is denoted by the presence of more than two cells with diffuse and intense activity, i.e., more than 40 granules.<sup>3</sup> To evaluate Golgi staining, characteristic of thymus derived lymphocytes (T-cells), Sigma-Aldrich Procedure No. 181 is recommended.<sup>4</sup>

Acid Phosphatase activity appears as purplish to dark red granules in the cytoplasm of most leukocytes.

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.

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

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## REFERENCES

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1. Goldberg AF, Barka T: Acid phosphatase activity in human blood cells. *Nature* 189:297, 1962
2. Janckila A, Li CY et al: The cytochemistry of the tartrate-resistant acid phosphatase-technical considerations. *Am J Clin Pathol* 70:45, 1978
3. Sun T: Atlas of Cytochemistry and Immunocytochemistry of Hematologic Neoplasms. American Society of Clinical Pathologists Press, Chicago, 1985, pp 28, 119
4. Starkweather WH, Small GJ, Hill Sk: A systematic approach to the cytochemical classification of acute leukemia. IN *Laboratory Perspectives*, Roger Maler, Inc., Arlington (NJ), issue No. 5, 1985, p 2

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