SIGMA-ALDRICH® ACID PHOSPHATASE, LEUKOCYTE

(Procedure No. 387)

INTENDED USE

Acid Phosphatase kit is intended for the cytologic demonstration of acid phosphatase in leukocytes from blood, bone marrow or tissue touch preparations. Acid Phosphatase reagents are for "In Vitro Diagnostics 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.[1] The Sigma-Aldrich procedure for demonstrating this enzyme employs naphthol AS-BI phosphate and freshly diazotized fast garnet GBC salt. The latter compound was selected because it couples rapidly at acid pH, forming highly insoluble dye deposits.

Most procedures, including that provided by Sigma-Aldrich, employ stable diazonium salts. These are formed by reacting an arylamine with sodium nitrite in an acid medium.[2] The resulting diazonium chloride (usually unstable) can then be treated with compounds such as zinc chloride, zinc sulfate or naphthalene-1,6-disulfonate, forming stable salts. These stabilizers may exert marked inhibition upon some enzymatic systems, whereas the diazonium chlorides are less inhibitory.[2] For this reason, Sigma-Aldrich now provides a stable solution of fast garnet GBC base and sodium nitrite for acid phosphatase cytochemistry. To further simplify this method, a stable aqueous solution of naphthol AS-BI phosphate is also included. The availability of these stable solutions allows users to adjust working reagent volumes according to needs, eliminating waste.

According to the Sigma-Aldrich procedure, blood films are incubated in a solution containing naphthol AS-BI phosphoric acid and freshly diazotized fast garnet GBC. Duplicate films may be treated with a solution containing L(+)-tartrate. 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. Those mononuclear cells containing tartaric acid-resistant phosphatase are not affected by such treatment.

REAGENTS

NAPHTHOL AS-BI PHOSPHORIC ACID SOLUTION, Catalog No. 3871-10 ml
Naphthol AS-BI phosphoric acid, 12.5 mg/ml.

FAST GARNET GBC BASE SOLUTION, Catalog No. 3872-10 ml
Fast garnet GBC base, 7.0 mg/ml, in 0.4 mol/l hydrochloric acid with stabilizer.

ACETATE SOLUTION, Catalog No. 3863-50 ml
Acetate buffer, 2.5 mol/l, pH 5.2 ± 0.1.

TARTRATE SOLUTION, Catalog No. 3873-10 ml
L(+)-Tartrate buffer, 0.335 mol/l, pH 4.9 ± 0.1.

SODIUM NITRITE SOLUTION, Catalog No. 914-10 ml
Sodium nitrite, 0.1 mol/l.

CITRATE SOLUTION, Catalog No. 915-50 ml
Citric acid, 18 mmol/l, sodium citrate, 9 mmol/l, sodium chloride, 12 mmol/l, and surfactant, pH 3.6 ± 0.1.

HEMATOXYLIN SOLUTION, GILL No. 3, Catalog No. GHS3-50 ml
Hematoxylin, certified, 6.0 g/l, sodium iodate, 0.6 g/l, aluminum sulfate, 52.8 g/l and stabilizers.

STORAGE AND STABILITY:

Store Hematoxylin Solution at room temperature (18–26°C). Protect from light. Reagent label bears expiration date.

Store Napthol AS-BI Phosphoric Acid Solution, Fast Garnet GBC Base Solution, Acetate Solution, Tartrate Solution, Sodium Nitrite Solution and Citrate Solution at 2–8°C.

Store Fixative Solution refrigerated (2–8°C). Warm to 18–26°C prior to use. Stable up to 2 months, if stored tightly capped in refrigerator. Discard Fixative Solution if evaporation is noted and prepare fresh.

DETERIORATION:

Naphthol AS-BI Phosphoric Acid Solution, Tartrate Solution should be discarded if turbidity develops.

Citrate Solution is suitable for use in the absence of microbial growth.

Hematoxylin Solution should be discarded if solution turns brown (over-oxidized from air) or purple (loss of acidity).

PREPARATION:

Reagents are provided ready for use.

Prepare Fixative Solution by combining 25 ml Citrate Solution, 65 ml acetone and 8 ml of 37% formaldehyde. Place in glass bottle and cap tightly.

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 and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. 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.[3] 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
Formaldehyde, 37%

NOTES:

Test performance should be monitored by including blood film prepared from a healthy individual.

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:

TEST PROCEDURE

1. Prewarm sufficient deionized water for a day's use to 37°C. Check temperature before use.
2. Bring Fixative Solution to room temperature (18–26°C). Fix slides by immersing in Fixative Solution for 30 seconds. Rinse thoroughly in deionized water: Do not allow slides to dry.
3. To each of 2 test tubes add 0.5 ml Fast Garnet GBC Base Solution and 0.5 ml Sodium Nitrite Solution. Mix by gentle inversion for 30 seconds. Let stand 2 minutes.
4. Label two 100-ml beakers A and B and add the following while mixing

	Beaker A	Beaker B
Deionized water prewarmed to 37°C	45 ml	45 ml
Diazotized Fast Garnet GBC Solution from Step 3	1.0 ml	1.0 ml
Naphthol AS-BI Phosphate Solution	0.5 ml	0.5 ml
Acetate Solution	2.0 ml	2.0 ml
Tartrate Solution	—	1.0 ml

5. Label Coplin jars A and B and transfer solutions from beakers to appropriate Coplin jar. Warm solutions in jars to 37°C in water bath. Check that temperature is at 37°C before adding slides.
6. Add slides to Coplin jars and incubate 1 hour in 37°C water bath protected from light.
7. After 1 hour, rinse slides thoroughly in deionized water, then counterstain 2 minutes in Hematoxylin Solution, Gill No. 3.
8. Rinse several minutes in alkaline tap water to blue nuclei.
9. Air dry and evaluate microscopically. Coverslipping is not recommended since dye fades with time.

PERFORMANCE CHARACTERISTICS

RESULTS:

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.[4] 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.[4] To evaluate Golgi staining, characteristic of thymus derived lymphocytes (T-cells), Sigma-Aldrich Procedure No. 181 is recommended.[5]

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

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

REFERENCES

1. Goldberg AF, Barka T: Acid phosphatase activity in human blood cells. Nature 189:297 1962
2. Burstone MS: In Enzyme Histochemistry and Its Application in the Study of Neoplasms. Academic Press, New York, 1962, pp 88–113
3. Janckila A, Li CY, et al: The Cytochemistry of the Tartrate-Resistant Acid Phosphatase – Technical considerations. Am J Clin Pathol 70:45, 1978
4. Sun T: Atlas of Cytochemistry and Immunochemistry of Hematologic Neoplasms. American Society of Clinical Pathologists Press, Chicago, 1985, pp 28, 119
5. 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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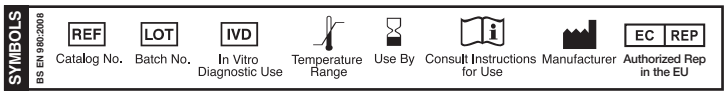

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