SIGMA-ALDRICH® LEUKOCYTE PEROXIDASE (MYELOPEROXIDASE)

(Procedure No. 390)

INTENDED USE

Sigma-Aldrich Leukocyte Peroxidase (Myeloperoxidase) is intended for use for histochemical demonstration in leukocyte peroxidase. Leukocyte Peroxidase reagents are for "In-Vitro Diagnostic Use."

Classic methods of cytochemical localization of myeloperoxidase (MP) have involved use of benzidine[1] or diaminobenzidine.[2] In 1977, Hanker et al.[3] described the use of p-phenylenediamine and catechol to detect injected horseradish peroxidase. That indicator system is the basis for the Sigma-Aldrich procedure when myeloperoxidase is detected by means of the following reaction:

p-Phenylenediamine + Catechol + H_2O_2 $\xrightarrow{MP}$ Brown-Black Insoluble Reaction Product

REAGENTS

TRIZMAL™ 6.3 BUFFER CONCENTRATE, Catalog No. 903C-50 ml
TRIZMA® maleate, 200 mmol/l. Chloroform added as preservative.
PEROXIDASE INDICATOR REAGENT, Catalog No. 3901-10 vl
p-Phenylenediamine diHCl (1 part) and catechol (2 parts).
ACID HEMATOXYLIN SOLUTION, Catalog No. 2852-100 ml
Hematoxylin, certified, 1 g/l, pH 3.3 at 25°C.
STORAGE AND STABILITY:

TRIZMAL™ 6.3 Buffer Concentrate and Acid Hematoxylin Solution should be stored at room temperature (18–26°C).

Peroxidase Indicator Reagent should be stored refrigerated (2–8°C).

Acid Hematoxylin Solution should not be returned to original container after use in Coplin jar.

Hydrogen Peroxide, 3% in Phosphate Buffered Saline Solution should be stored in refrigerator (2–8°C). Discard if turbidity develops.

Reagents are stable until expiration date.

DETERIORATION:

Discard TRIZMAL™ 6.3 Buffer Concentrate if turbidity develops.

Discard Acid Hematoxylin Solution when the time required for suitable staining exceeds the time recommended in the procedure by more than 5 minutes.

PREPARATION:

TRIZMAL™ 6.3 Dilute Buffer is prepared by mixing 1 volume of TRIZMAL™ 6.3 Buffer Concentrate with 9 volumes of deionized water. Use once and discard.

Fixative Solution is prepared by mixing 5 ml of 37% formaldehyde with 45 ml of 95% ethanol. Prepare fresh daily. Store tightly capped.

Hydrogen Peroxide, 3%, in Phosphate Buffered Saline Solution, is prepared by adding 1 part Hydrogen Peroxide, 30%, to 9 parts Phosphate Buffered Saline Solution
pH 7.4. Should be prepared fresh.

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.

Freshly prepared whole blood or bone marrow films should be used for the assay. Blood may be collected in heparin or EDTA. Exposure to light should be minimized as leukocyte peroxidase is photolabile. Unfixed films are reported to be stable for 3 weeks when kept in the dark.[1] Allow films to air dry for 10 minutes, protected from light, prior to fixation.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
Formaldehyde, 37%, Solution
Ethanol, 95% (v/v)
Phosphate Buffered Saline, pH 7.4, Catalog No. P 3813
Hydrogen Peroxide, 30%

NOTES:

It is recommended that blood films prepared from healthy donors be processed along with patient samples as a system performance check.

Although myeloperoxidase is generally considered a marker for cells of myelocytic lineage, it is imperative to recognize that monocytoid cells may also display weak peroxidase 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:

1. Fix films at room temperature for 30 seconds in Fixative Solution.
2. Wash slides in gently running tap water for 2 minutes and allow to air dry in the dark for 10 minutes.
3. Prewarm 50 ml TRIZMAL™ 6.3 Dilute Buffer in 37°C water bath.
4. Immediately before use, add 1 vial Peroxidase Indicator Reagent and 200 µl (0.2 ml) of 3% Hydrogen Peroxide to the prewarmed TRIZMAL™ 6.3 Dilute Buffer. Mix thoroughly. Discard after use.
5. Place washed, fixed slides (Step 2) in Peroxidase Indicator Reagent Solution (Step 4) for 30 minutes in the dark in 37°C water bath.
6. Following incubation, wash slides in gently running tap water for 15–30 seconds and allow to air dry.
7. Counterstain slides in Acid Hematoxylin Solution for 10 minutes.
8. Rinse slides in running deionized water for 15–30 seconds. Air dry and examine slides microscopically.

PERFORMANCE CHARACTERISTICS

Blood films prepared from normal donors were stained for myeloperoxidase according to this procedure and by a benzidine method.[1] Neutrophils showed brown-black granulation with this procedure and blue granulation with the benzidine procedure. In both cases, monocytes stained less intensely and lymphocytes did not show myeloperoxidase activity.

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

REFERENCES

1. Kaplow SL: Simplified myeloperoxidase stain using benzidine dihydrochloride. Blood 26:215, 1965.
2. Graham RC, Karnovsky MJ: The early stage of absorption of injected horseradish peroxidase in the proximal tubule of mouse kidney; Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291, 1966.
3. Hanker JS, Yates PE, Metz CB, Rustioni A: A new specific sensitive and non-carcinogenic reagent for the demonstration of horseradish peroxidase. Histochem 9:789, 1977.
4. Bennett JM, Catovsky D, Daniel M, Randrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of the acute leukemias. French-American-British (FAB) Co-operative Group. Brit J Haematol 33:451, 1976.
5. Marmont AM, Damasio E, Zucker-Franklin D: Neutrophils. In Atlas of Blood Cells-Function and Pathology, Vol. 1. Edited by D Zucker-Franklin, MF Greaves, CE Grossi, AM Marmont, Lea and Febriger, Philadelphia, 1981, pp 149–424.

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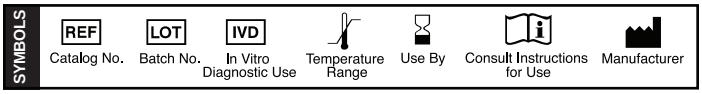

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