SIGMA-ALDRICH®

PEROXIDASE (MYELOPEROXIDASE)

(Procedure No. 391)

INTENDED USE

Peroxidase (Myeloperoxidase) kit is a cytochemical staining system for polymorphonucleocytes in blood or bone marrow films. The staining characteristics of polymorphonucleocytes are used to distinguish acute myelocytic leukemia from other types of leukemia. Peroxidase reagents are for "In-Vitro Diagnostic Use."

Diaminobenzidine (DAB), as a benzidine substitute for peroxidase (myeloperoxidase) cytochemistry, has less tinctorial power than benzidine.[1,2] This made DAB much less attractive to histochemists. However, Hanker and associates improved DAB methodology, making it more suitable for differentiating granulocytes, their precursors and monocytes from cells of lymphoid origin.[3-6] According to their modification, the brown reaction product is first intensified with copper salts followed by application of Gill's modified Papanicolaou stain, resulting in intense grey-black granules at sites of neutrophil and monocyte myeloperoxidase.[7]

With the Hanker DAB reaction, samples from acute myelocytic leukemia (AML) patients exhibit more spindle or fusiform shaped rods (phi bodies) than when the conventional DAB system is used. This could mean the Hanker DAB method is more sensitive for detection of AML. The Sigma-Aldrich procedure is similar to that developed by Hanker, involving the following reactions:

$$DAB + H_2O_2 \xrightarrow{MP} \text{Oxidized DAB (Light Brown Pigment)}$$

$$\text{Oxidized DAB} + Cu(NO_3)_2 \longrightarrow \text{Grey-Black Pigment}$$

When treated with Gill's modified Papanicolaou stain, the reaction product is further intensified and characteristic color imparted to neutrophils, eosinophils and basophils. It should be noted that evidence indicating the carcinogenicity of DAB is equivocal and good laboratory practice should preclude any potential hazard.[8]

REAGENTS

DIAMINOBENZIDINE, Catalog No. 3911-1vl
Diaminobenzidine tetrahydrochloride, 25 mg/vial (10 vials)

COPPER NITRATE, Catalog No. 3912-1vl
Copper nitrate, 625 mg/vial (2 vials)

TRIZMA® BUFFER CONCENTRATE, Catalog No. 3913-50 ml
TRIZMA®-HCl buffer, 1.0 mol/l.

GLUTARALDEHYDE SOLUTION, Catalog No. 3802-75 ml
Glutaraldehyde, 4%, and borate buffer, 67 mmol/l, pH 7.6. (2x75 ml)

HEMATOXYLIN SOLUTION, GILL NO. 3, Catalog No. GHS3-100 ml
Hematoxylin (certified), 6 g/l, sodium iodate, 0.6 g/l, aluminum sulfate, 52.8 g/l, and stabilizer.

GILL MODIFIED EA SOLUTION, Catalog No. 3915-100 ml
Fast Green FCF (certified), 0.017% (w/v), Eosin Y (certified), 0.4% (w/v), alcohol, 73% (v/v), absolute methyl alcohol, 25% (v/v), glacial acetic acid, 2% (v/v) and phosphotungstic acid, 0.4% (w/v).

SCOTT'S TAP WATER SUBSTITUTE CONCENTRATE, Catalog No. S5134-100 ml
Contains magnesium sulfate•$7H_2O$, 200 g/l, sodium bicarbonate, 20 g/l, and preservative.

STORAGE AND STABILITY:

Store Diaminobenzidine and Glutaraldehyde Solution in refrigerator (2–8°C). Reagent labels bear expiration date. Store other kit reagents at room temperature (18–26°C). Protect Hematoxylin Solution from light. Reagent labels for Copper Nitrate and Gill Modified EA Solution bear expiration date.

Store Copper Nitrate Solution and Scott's Tap Water Substitute Working Solution at room temperature (18–26°C). Store TRIZMA® Working Solution in refrigerator (2–8°C). If turbidity develops, discard Copper Nitrate Solution, TRIZMA® Buffer Concentrate and Working Solution, Scott's Tap Water Substitute Concentrate and Working Solution.

Store Glutaraldehyde-Acetone Fixative tightly stoppered in refrigerator (2–8°C). Stable provided pH is in range 7.2 to 8.0.

DETERIORATION:

Discard Hematoxylin Solution, Gill No. 3, if solution turns brown (air oxidation) or purple (loss of acidity).

PREPARATION:

Copper Nitrate Solution is prepared by dissolving contents of 1 vial Copper Nitrate in 250 ml deionized water.

TRIZMA® Working Solution (pH 7.6 ± 0.3) is prepared by diluting 1 volume of TRIZMA® Buffer Concentrate with 9 volumes deionized water.

Glutaraldehyde-Acetone Fixative Solution is prepared by adding 25 ml reagent grade acetone to 75 ml Glutaraldehyde Solution.

Scott's Tap Water Substitute Working solution is prepared by diluting 1 volume Scott's Tap Water Substitute Concentrate with 9 volumes deionized water.

Filter Hematoxylin Solution, Gill No. 3, before 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 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.

Bone marrow aspirate, peripheral blood or buffy coat preparations may be used. Either heparin or EDTA serves as a satisfactory anticoagulant.[3] Unfixed samples may be stored at room temperature (18–26°C) protected from light for several months without loss of activity.[4]

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Ethanol, 95% and Absolute
Hydrogen Peroxide, 1%
Acetone, ACS Reagent
Xylene or xylene substitute

NOTES:

It is recommended that blood films prepared from healthy donors be processed along with patient samples to monitor system performance.

Although myeloperoxidase is generally considered a marker for cells of myelocytic lineage, it is imperative to recognize that monoytoid 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. Dissolve contents of 1 Diaminobenzidine vial in 50 ml TRIZMA® Working Solution.
2. Set up a series of staining jars containing the following:
   a. Glutaraldehyde-Acetone Fixative Solution
   b. Diaminobenzidine Solution prepared in Step 1
   c. Copper Nitrate Solution
   d. Hematoxylin Solution, Gill No. 3.
   e. Scott's Tap Water Substitute Working Solution
   f. Gill's Modified EA Solution
   g. Two dishes with 95% ethanol
   h. Two dishes with absolute ethanol
   i. Three dishes with xylene See NOTE following Step 18
3. Immediately prior to fixing slides, add 0.5 ml 1% hydrogen peroxide to Diaminobenzidine solution prepared in Step 1. Mix well.
4. Fix slides at 4–8°C in Glutaraldehyde-Acetone Fixative Solution for 1 minute.
5. Rinse briefly (30 seconds) in deionized water.
6. Incubate for 45 seconds in Diaminobenzidine/Peroxide solution prepared in Step 3.
7. Rinse briefly (30 seconds) in deionized water.
8. Immerse in Copper Nitrate Solution for 2 minutes with gentle agitation.
9. Rinse briefly (30 seconds) in deionized water.
10. Immerse in Hematoxylin Solution, Gill No. 3 for 8 seconds (4 dips).
11. Rinse in 2 changes of deionized water for 5 seconds with agitation.
12. Immerse for 12 seconds (6 dips) in Scott's Tap Water Substitute Working Solution.
13. Rinse in 2 changes of deionized water for 5 seconds.
14. Immerse in Gill Modified EA Solution for 1 minute.
15. Rinse in 2 changes of 95% ethanol for 3 seconds each.
16. Rinse in 2 changes absolute ethanol for 3 seconds each.
17. Rinse in 3 changes xylene for 3 seconds each.
18. Mount in permanent mounting media and examine microscopically. Color will fade if slides are not mounted.

    NOTE: The procedure may be shortened by eliminating Steps 14–17. Eosinophil peroxidase will appear dark red-brown instead of red-orange. If Gill Modified EA Solution is used, rinsing in alcohol and xylene must be included.

PERFORMANCE CHARACTERISTICS

Neutrophils and their precursors show grey-black intracellular granulation. Monocytes stain less intensely. Eosinophils stain red-orange while basophils stain blue. Lymphocytes do not show peroxidase activity.

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.

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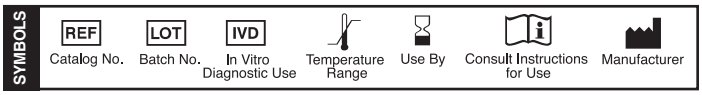

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