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.” amino-benzidine (DAB), as a benzidine substitute for peroxidase (myeloperoxidase) cytochemistry, has less toxic potential than benzidine.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.4 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.5

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:

\[
\text{DAB} + H_2O_2 \rightarrow \text{Oxidized DAB (Light Brown Pigment)}
\]

\[
\text{Oxidized DAB} + \text{Cu(NO}_3\text{)}_2 \rightarrow \text{Grey-Black Pigment}
\]

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.6

REAGENTS

DIAMINOBENZIDINE, Catalog No. 3911-1v1
Diaminobenzidine tetrahydrochloride, 25 mg/vial (10 vials)
COPPER NITRATE, Catalog No. 3912-1v1
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), 0.0171% (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. 55134-100 ml
Contains magnesium sulfate•7H2O, 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.

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. 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
4. Hanker JS, Ambrose WW, James CJ, et al: Facilitated light microscopic cytochemistry, has less tinctorial power than benzidine. Blood films prepared from normal donors were stained for myeloperoxidase according to this procedure and by a benzidine method. 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.
TRIZMA is a registered trademark of Sigma-Aldrich Co. LLC

2014 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.

Procedure No. 391
Previous Revision: 2003-09
Revised: 2014-09