SIGMA-ALDRICH®

NAPHTHOL AS-D CHLOROACETATE ESTERASE AND α-NAPHTHYL ACETATE ESTERASE

(Procedure No. 90)

INTENDED USE

Sigma-Aldrich reagents are intended for the cytologic demonstration of naphthol AS-D chloroacetate esterase and α-naphthyl acetate esterase in blood, bone marrow films or tissue touch preparations. Esterase reagents are for "In Vitro Diagnostic Use".

Cellular esterases are ubiquitous and appear to represent a series of different enzymes acting upon select substrates. Under defined reaction conditions, it may be possible to determine hemopoietic cell types, using specific esterase substrates. The described methods provide means to distinguish granulocytes from monocytes.[1-8]

To perform the test, blood, bone marrow films or tissue touch preparations are incubated with either naphthol AS-D chloroacetate or α-naphthyl acetate in the presence of a stable diazonium salt. Enzymatic hydrolysis of ester linkages liberates free naphthol compounds. These couple with the diazonium salt, forming highly colored deposits at the sites of enzyme activity.

REAGENTS

DIMETHYL FORMAMIDE, Catalog No. 9010-25 ml

ETHYLENE GLYCOL MONOMETHYL ETHER,
Catalog No. 9011-25 ml

NAPHTHOL AS-D CHLOROACETATE,
Catalog No. 905-10CAP
Capsule contains 20 mg.

α-NAPHTHYL ACETATE, Catalog No. 906-10CAP
Capsule contains 20 mg.

TRIZMAL™ 6.3 BUFFER CONCENTRATE,
Catalog No. 903C-50 ml
TRIZMA® maleate, 200 mmol/l. Chloroform added as preservative.

TRIZMAL™ 7.6 BUFFER CONCENTRATE,
Catalog No. 902C-50 ml
TRIZMA® maleate, 200 mmol/l. Chloroform added as preservative.

MAYER S HEMATOXYLIN SOLUTION, Catalog No. MHS1-100 ml
Hematoxylin, certified, 0.1% (w/v), and stabilizers.

ACID HEMATOXYLIN SOLUTION, Catalog No. 2852-100 ml
Hematoxylin certified, 1 g/l, and stabilizers, pH 3.3 at 25°C.

FAST BLUE RR SALT, Catalog No. FBS25-10CAP
Preweighed capsules. Actual weight per capsule will vary with dye lot purity and has been optimized by assay.

FAST CORINTH V SALT, Catalog No. 9015-10CAP

CITRATE CONCENTRATE, Catalog No. 3861-20 ml
Citrate buffer, 0.383 mol/l, pH 5.4 when diluted according to procedure.

STORAGE AND STABILITY:

Dimethyl Formamide, Ethylene Glycol Monomethyl Ether, TRIZMAL™ 6.3 Buffer Concentrate, TRIZMAL™ 7.6 Buffer Concentrate, Mayer's Hematoxylin Solution and Acid Hematoxylin Solution, are stored at room temperature (18–26°C).

Naphthol AS-D Chloroacetate, α-Naphthyl Acetate and Fast Blue RR Salt are stored below 0°C.

Fast Corinth V Salt and Citrate Concentrate are stored refrigerated (2–8°C).

Naphthol AS-D Chloroacetate, α-Naphthyl Acetate, Fast Blue RR Salt and Fast Corinth V Salt are stable until the expiration date shown on the labels.

Citrate Dilute Solution is stable for 1 week if stored tightly capped at room temperature (18–26°C).

TRIZMAL™ 6.3 Buffer Concentrate, TRIZMAL™ 7.6 Buffer Concentrate and Citrate Concentrate are suitable for use in the absence of microbial growth.

Sodium fluoride, 2 g/dl. Store at room temperature (18–26°C). Used if "α-Naphthyl Acetate Esterase with Fluoride Inhibition Procedure" is performed.

DETERIORATION:

Discard Dimethyl Formamide and Ethylene Glycol Monomethyl Ether if colored or turbid.

TRIZMAL™ 6.3 Dilute Buffer Solution and TRIZMAL™ 7.6 Dilute Buffer Solution should be used once then discarded.

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

PREPARATIONS:

NAPHTHOL AS-D CHLOROACETATE SOLUTION is prepared by dissolving contents of 1 capsule Naphthol AS-D Chloroacetate in 2 ml Dimethyl Formamide. Remove 1 capsule from freezer as needed. Prepare immediately prior to use.

α-NAPHTHYL ACETATE SOLUTION is prepared by dissolving contents of 1 capsule α-Naphthyl Acetate in 2 ml Ethylene Glycol Monomethyl Ether. Remove 1 capsule from freezer as needed. Prepare immediately prior to use.

TRIZMAL™ 6.3 DILUTE BUFFER SOLUTION is prepared by diluting 1 part TRIZMAL™ 6.3 Buffer Concentrate with 9 parts deionized water. The pH should be 6.3 at 25°C.

TRIZMAL™ 7.6 DILUTE BUFFER SOLUTION is prepared by diluting 1 part TRIZMAL™ 7.6 Buffer Concentrate with 9 parts deionized water. The pH should be 7.6 at 25°C.

Mayer's Hematoxylin and Acid Hematoxylin Solution should be filtered before use.

CITRATE DILUTE SOLUTION is prepared by diluting 1 part Citrate Concentrate with 9 parts deionized water. pH 5.4 when diluted.

CITRATE-ACETONE-METHANOL FIXATIVE: To 18 ml Citrate Dilute Solution, add 27 ml ACS grade Acetone and 5 ml Methanol. Store tightly capped at room temperature (18–26°C). Discard after 8 hours.

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 with both α-naphthyl acetate esterase and naphthol AS-D chloroacetate esterase. Either EDTA or heparin will serve as an anticoagulant.[9] Frozen and paraffin embedded tissues may be used with naphthol AS-D chloroacetate esterase. α-Naphthyl acetate esterase may be used successfully on frozen tissue sections.[10] 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.[5,9] 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:

Methanol, Absolute

Acetone, ACS Reagent

Sodium Fluoride Solution, Catalog No. 919-25 ml

Sodium fluoride, 2 g/dl

NOTES:

The described procedures are performed at 37°C. If reagents are not at this temperature, weak or negative reactions may be obtained. It is recommended that temperatures be checked with an accurate thermometer. Controlled temperature water baths are more efficient than warm air incubators and should be used for enzyme cytochemical methods. Heat transfer through glass is more rapid than through plastic, thus, glass Coplin jars should be employed.

Many enzyme systems are sensitive to minute traces of detergent. Washing glassware with dilute bleach followed by rinsing in copious quantities of deionized water will prevent detergent effect upon cellular enzymes.

Results are based on a certain degree of subjective interpretation. Individual laboratories should establish their own normal ranges.

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:
NAPHTHOL AS-D CHLOROACETATE ESTERASE PROCEDURE:

1. Fix slides for 1 minute in Citrate-Acetone-Methanol Fixative at room temperature (18–26°C).
2. Wash thoroughly in deionized water and air dry at least 20 minutes.
3. To 50 ml TRIZMAL™ 6.3 Dilute Buffer Solution, PREWARMED TO 37°C, add with constant stirring, contents of 1 capsule Fast Corinth V Salt.
4. When salt is completely dissolved in buffer, add 2 ml Naphthol AS-D Chloroacetate Solution. The solution will appear quite turbid.
5. Continue mixing for 15–30 seconds, then add to Coplin jar. DO NOT FILTER.
6. Place specimens in staining solution (from Step 5) and incubate at 37°C for 5 minutes. NOTE: PROTECT FROM LIGHT.
7. Remove slides from stain and wash in deionized water for 3 minutes. Discard staining solution.
8. If desired, counterstain in Acid Hematoxylin Solution for 5–10 minutes and wash in tap water.
9. Air dry slides and examine microscopically. If coverslipping is required use only an aqueous mounting media.

α-NAPHTHYL ACETATE ESTERASE PROCEDURE:

1. Fix slides in Citrate-Acetone-Methanol Fixative for 1 minute at room temperature (18–26°C).
2. Wash thoroughly in deionized water and air dry at least 20 minutes.
3. To 50 ml TRIZMAL™ 7.6 Dilute Buffer Solution, PREWARMED TO 37°C, add with constant stirring, contents of 1 capsule Fast Blue RR Salt.
4. When salt is completely dissolved in buffer, add 2 ml α-Naphthyl Acetate Solution. The solution will be yellow and slightly turbid.
5. Continue stirring for 15–20 seconds, then add to Coplin jar. DO NOT FILTER.
6. Place specimens in staining solution (from Step 5) and incubate at 37°C for 30 minutes.
NOTE: PROTECT FROM LIGHT.
7. Remove slides from stain and wash for 3 minutes in deionized water. Discard staining solution.
8. If desired, counterstain for 5–10 minutes in Mayer's Hematoxylin Solution, and wash in tap water.
9. Air dry slides and examine microscopically. If coverslipping is required use only an aqueous mounting media.

DOUBLE STAINING ESTERASE PROCEDURE

1. Perform α-Naphthyl Acetate Esterase test as described in Procedure. Do not counterstain.
2. Rinse slides 5 minutes in deionized water.
3. Perform Naphthol AS-D Chloroacetate Esterase test as described in procedure Steps 3-9.

α-NAPHTHYL ACETATE ESTERASE WITH FLUORIDE INHIBITION PROCEDURE

Although α-naphthyl acetate esterase is found primarily in cells of monocytic lineage when performed as described, it should be recognized that megakaryocytes and erythroid precursors are positive for this enzyme.[11] Lymphocytes and some mature granulocytes also show occasional positivity.[5] To differentiate these cells conclusively from monocytes, sodium fluoride is incorporated with the incubation system. The monocyte enzyme is inactivated in the presence of this compound.[12] The following procedure may be used to perform the fluoride inhibition test.

1. Fix slides in Citrate-Acetone-Methanol Fixative for 1 minute at room temperature (18–26°C).
2. Wash thoroughly in deionized water and air dry at least 20 minutes.
3. Label 2 beakers A and B, and add the following:

	Beaker A	Beaker B
Prewarmed 37°C TRIZMAL™ 7.6 Dilute Buffer	50 ml	50 ml
Add with constant stirring, Fast Blue RR	1 capsule*	1 capsule*
α-Naphthyl Acetate Solution	2 ml	2 ml
Sodium Fluoride Solution	—	2 ml

*Contents of 1 capsule

4. Mix well and pour into Coplin jars labeled A and B.
5. Proceed as described in Steps 6–9 of α-Naphthyl Acetate Esterase Procedure.

PERFORMANCE CHARACTERISTICS

METHOD OF SCORING:

Scan the film and select a thin area with few erythrocytes. Sites of Naphthol AS-D Chloroacetate Esterase activity will appear as bright red granulation, α-Naphthyl Acetate Esterase as black granulation. Rate from 0 to 4 + on the basis of quantity and intensity of individual dyes within the cytoplasm of the respective cell types. Characteristics of scoring are based somewhat on subjective interpretation. A suggested scoring format is presented in Table 1. Conclusions center on relative presence or absence of staining.

TABLE I
Characteristics of Scoring

Cell Rating	Intensity of Staining	Interpretation
0	None	–
1+	Faint to Moderate	±
2+	Moderate to Strong	+
3+	Strong	+
4+	Brilliant	+

RESULTS:

NAPHTHOL AS-D CHLOROACETATE ESTERASE

This enzyme is usually considered specific for cells of granulocytic lineage. The cells should show red granulation. Activity is weak or absent in monocytes and lymphocytes.

α-NAPHTHYL ACETATE ESTERASE

Under the assay conditions (pH 7.6), this enzyme is detected primarily in monocytes, macrophages and histiocytes, and is virtually absent in granulocytes. Monocytes should show black granulation. Lymphocytes may occasionally exhibit activity.

α-NAPHTHYL ACETATE ESTERASE WITH FLUORIDE INHIBITION

All cells of monocytic lineage will be negative for enzyme activity, with the exception of differentiated histiocytes or specialized macrophages in tissue which may also be resistant to sodium fluoride.[10]

DOUBLE STAINING ESTERASE

Specimens taken through the double staining procedure will demonstrate the granulocytes with red granulation and monocytes with black granulation.

The expected cellular reactivity of tests for esterase activity is summarized in Table II.

TABLE II
Cytochemical Reactions of Blood Cells

Cell Type	Naphthol AS-D Chloroacetate Esterase	α-Naphthyl Acetate Esterase
Myeloblasts	±	±
Promyelocytes	+	±
Neutrophils	+	—
Eosinophils	—	—
Basophils	±	—
Monocytes	—	+
Lymphocytes	—	±
Lymphoblasts	—	±
Megakaryocytes	—	+
Erythroblasts	—	±
Plasma Cells	—	±
Mast Cells	+	—
Hairy Cells	—	±
Histiocytes	±	+

The reagent system should be monitored by the use of positive and negative control slides. Positive control slides may be prepared from leukemic specimens or specific cell lines known to be positive.

Alternately, anti-coagulated blood from normal specimens (preferably with increased monocyte count if using α-naphthyl acetate esterase procedure) may also be used; however, they will provide less intense staining and will have fewer positive cells.

Known negative patient slides may be used as a negative control. If unavailable, staining a specimen in an incubation mixture with the substrate omitted will give the desired results. However, use of the former is highly recommended.

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

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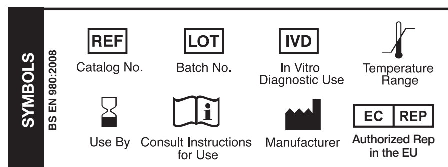

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