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 hematopoietic cell types, using specific esterase substrates. The described methods provide means to distinguish granulocytic from monocytes.

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
TRIZMAL™ maleate, 200 mMol/ml. Chloroform added as preservative.
TRIZMAL™ 7.6 BUFFER CONCENTRATE, Catalog No. 902C-50 ml
TRIZMAL™ maleate, 200 mMol/ml. Chloroform added as preservative.
MAYER’S HEMATOXYLIN SOLUTION, Catalog No. MHS1-100 ml
Mayer’s Hematoxylin and Acid Hematoxylin Solution, are stored at room temperature (18–26°C).

NOTE: PROTECT FROM LIGHT.

MAYER’S HEMATOXYLIN SOLUTION, Catalog No. MHS1-100 ml
Mayer’s Hematoxylin and Acid Hematoxylin Solution, are stored at room temperature (18–26°C).

PHYSICAL PROPERTIES

НАPHTHOL AS-D CHLOROACETATE ESTERASE

- pH: 5.4 when diluted.
- Sodium fluoride is incorporated with the incubation system. The enzyme activity is affected by fluoride, and the activity is inhibited. This is commonly used to inhibit enzyme activity. The enzyme activity is also commonly used in the presence of sodium fluoride to inhibit enzyme activity.

α-NAPHTHYL ACETATE ESTERASE

- pH: 7.6
- IF desired, counterstain for 5–10 minutes in Mayer’s Hematoxylin Solution, and wash in tap water.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can provide complete assurance that blood specimens or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Blood, bone marrow films, tissue-tissue 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.† Frozen and paraffin embedded tissues may be used with naphthol AS-D chloroacetate esterase. α-Naphthyl acetate esterase may be used successfully on frozen sections.‡ Blood or bone marrow films may be stored fixed at room temperature (18–26°C) for several weeks or unfixed for several minutes without appreciable change in activity.‡ Do not ship whole blood for assay at other laboratories. Send fixed or unfixed slides. Slides should be air dried before staining. 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/ml

NOTES:

The described procedures are performed at 37°C. If reagents are available at room temperature (18–26°C), †Do not ship whole blood for assay at other laboratories. Send fixed or unfixed slides. Slides should be air dried before staining. Allow films to dry at least 1 hour prior to fixation.

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. To 50 ml TRIZMAL™ 6.3 Dlute 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 for 5–10 minutes in Mayer’s Hematoxylin Solution, and wash in tap water.
9. Air dry slides and examine microscopically. If covenstaining 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™ 6.3 Dlute Buffer Solution, PREWARMED TO 37°C, add with constant stirring, contents of 1 capsule Fast Corinth V Salt.
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7. Remove slides from stain and wash in deionized water for 3 minutes. 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 covenstaining 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 macrophages and erythroid precursors are positive for this enzyme.1 Lymphocytes and some mature granulocytes also show occasional positivity. To differentiate these cells conclusively from monocytes, sodium fluoride is incorporated with the incubation system. The monocytic enzyme is inhibited in the presence of this compound. 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:

<table>
<thead>
<tr>
<th>Beaker A</th>
<th>Beaker B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Fluoride Solution — 2 ml</td>
<td>Sodium Fluoride Solution — 2 ml</td>
</tr>
<tr>
<td>Sodium Fluoride Solution — 2 ml</td>
<td>Sodium Fluoride Solution — 2 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Add with constant stirring, Fast Blue RR</th>
<th>Add with constant stirring, Fast Blue RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 capsule*</td>
<td>1 capsule*</td>
</tr>
</tbody>
</table>

*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.
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

<table>
<thead>
<tr>
<th>Characteristics of Scoring</th>
<th>Intensity of Staining</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>–</td>
</tr>
<tr>
<td>1+</td>
<td>Faint to Moderate</td>
<td>±</td>
</tr>
<tr>
<td>2+</td>
<td>Moderate to Strong</td>
<td>+</td>
</tr>
<tr>
<td>3+</td>
<td>Strong</td>
<td>+</td>
</tr>
<tr>
<td>4+</td>
<td>Brilliant</td>
<td>+</td>
</tr>
</tbody>
</table>

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.14

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

<table>
<thead>
<tr>
<th>Cytochemical Reactions of Blood Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Type</td>
</tr>
<tr>
<td>Myeloblasts</td>
</tr>
<tr>
<td>Promyelocytes</td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Eosinophils</td>
</tr>
<tr>
<td>Basophils</td>
</tr>
<tr>
<td>Monocytes</td>
</tr>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Lymphoblasts</td>
</tr>
<tr>
<td>Megakaryocytes</td>
</tr>
<tr>
<td>Erythroblasts</td>
</tr>
<tr>
<td>Plasma Cells</td>
</tr>
<tr>
<td>Mast Cells</td>
</tr>
<tr>
<td>Hairy Cells</td>
</tr>
<tr>
<td>Histiocytes</td>
</tr>
</tbody>
</table>

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.

### REFERENCES


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