SIGMA-ALDRICH® LYMPHOCYTE ENZYMES

(Procedure No. 181)

INTENDED USE

Lymphocyte Enzyme Kits are for use in the detections of acid phosphatase (Kit No. 181A) or α-naphthyl butyrate esterase (Kit No. 181B) in blood, bone marrow films and tissue touch preparations. Lymphocyte Enzyme Kits are for "In Vitro Diagnostic Use."

Dissection of normal T-cell maturation into discrete stages by enzyme cytochemistry and monoclonal antibodies has shown that T-cell malignancies mirror the same ontogenetic diversity.[1–14] These reports, in most part, support the conclusion that T-cell neoplasias reflect maturation arrest during normal development.

The OKT® series of monoclonal antibodies recognizing T-cell surface antigens delineate prothymocytes/thymocytes from mature T-cells. Similarly, most T-cells in peripheral blood and lymphatic tissue, when stained for enzymes such as acid phosphatase (AcP) and α-naphthyl butyrate esterase (α-NB), display characteristic age-related cytochemical profiles. AcP is acquired by early fetal thymocytes and is retained throughout T-cell differentiation.[15–16] An epitope of α-NB, reactive over a narrow pH range (5.7–6.0) is expressed by mature T-cells and medullary thymocytes.[18–20] From their results, Basso et al.,[16] have proposed a maturation scheme whereby differentiating T-cells progress from AcP+, BG-, α-NB- to AcP+, BG+, α-NB- and finally AcP+, BG+, α-NB+.

The majority of mature T-cells, as defined by sheep red blood cell rosetting, display a distinct focal (dot) pattern when stained for AcP and α-NB[21,22] and express surface receptors for IgM (Fcr).[23] Those characterized by a diffuse/granular stain express Fc receptors for IgG (Tg, gFcr).[9,23] There is some evidence that the subsets may overlap the OKT-4/OKT-8 (helper-suppressor) subsets.[17–20] Discordant data concerning this relationship have been reported.[9,23,24] At present, it appears that enzyme phenotypes assist primarily in determining the stage of maturation arrest in T-cell malignancies, and allow differentiation between T-, B-, and non-T/non-B lymphoproliferative disorders.

The described procedures allow dissection of the T-cell compartment into two discrete phases of development. These techniques do not obviate use of monoclonal antibodies for phenotypic analysis but, used in concert, may supply added information concerning the nature of T-cell lymphoproliferative disorders.

The reaction listed below for α-Naphthyl Butyrate Esterase will also demonstrate nonspecific esterase activity in monocytes and macrophages. Several different methods are available for this purpose. The method listed below is not specific for monocytes and macrophages since the pH of the incubating media has been adjusted to demonstrate a focal (dot) staining pattern in some lymphocytes. The α-Naphthyl Butyrate Esterase is less sensitive than the α-Naphthyl Acetate Esterase procedure. For procedures more sensitive to monocytes Sigma offers two Esterase kits, Catalog Nos. 90-A1 and 91-A. These kits also include instructions for the demonstration of α-naphthyl acetate esterase with fluoride inhibition.

According to Sigma-Aldrich techniques, cytocentrifuge preparations or films are fixed in a citrate-acetone-formaldehyde solution. Acid phosphatase, α-naphthyl butyrate esterase and ß-glucuronidase are then visualized by the following simultaneous capture principles.

Acid Phosphatase:

Naphthol AS-BI Phosphate —(AcP, GBC)→ Insoluble-Chromogenic Naphthol AS-BI-GBC Complex (Red-Violet)

α-Naphthyl Butyrate Esterase:

α-Naphthyl Butyrate —(α-NB, Hexazotized Pararosaniline)→ Insoluble-Chromogenic α-Naphthyl-HPR Complex (Red-Brown)

REAGENTS

α-NAPHTHYL BUTYRATE SOLUTION, Catalog No. 1801-50 ml
α-Naphthyl Butyrate, 2.4 g/l, in methanol solution with solubilizers.

NAPHTHOL AS-BI PHOSPHORIC ACID SOLUTION,
Catalog No. 1802-15 ml
Naphthol AS-BI phosphoric acid, 4 g/l, in methanol solution with solubilizers.
Naphthol AS-BI ß-D-glucuronic acid, 2.5 g/l, in methanol solution with solubilizers.

PARAROSANILINE SOLUTION, Catalog No. 1804-15 ml
Pararosaniline, 40 g/l, in 2 mol/l hydrochloric acid.

FAST GARNET GBC BASE SOLUTION, Catalog No. 3872-3 ml
Fast garnet GBC base, 7.0 mg/ml, in 0.4 mol/l hydrochloric acid and stabilizer.

SODIUM NITRITE SOLUTION, Catalog No. 914-10 ml
Sodium nitrite, 0.1 mol/l.

SODIUM NITRITE TABLETS, Catalog No. 1809-10TAB
Sodium nitrite, 250 mg per tablet.

CITRATE SOLUTION, Catalog No. 915-50 ml
Citric acid, 18 mmol/l, sodium citrate, 9 mmol/l, sodium chloride, 12 mmol/l, and surfactant. The pH should be 3.6 ± 0.1.

ACETATE SOLUTION, Catalog No. 3863-50 ml
Acetate buffer, 2.5 mol/l, pH 5.2.

PHOSPHATE BUFFER, Catalog No. 1805-1vL
Sodium and potassium phosphates.

METHYLENE BLUE SOLUTION, Catalog No. 1808-50 ml
Methylene blue, 1.4% (w/v) in 95% ethanol.

STORAGE AND STABILITY:

Store α-Naphthyl Butyrate Solution, Naphthol AS-BI Phosphoric Acid Solution in freezer below 0°C. Warm solutions to 37°C and mix well prior to use. Discard if reagents turn yellow or if precipitate forms.

Store Pararosaniline Solution, Phosphate Buffer, Sodium Nitrite Tablets and Methylene Blue Solution at room temperature (18–26°C) protected from light.

Store Fast Garnet GBC Base Solution, Sodium Nitrite Solution, Sodium Nitrite Tablet Solution, Acetate Solution and Phosphate Buffer Solution refrigerated (2–8°C).

Reagent labels bear expiration date.

Sodium Nitrite Solution and Citrate Solution are suitable for use in the absence of microbial growth.

DETERIORATION:

Discard α-Naphthyl Butyrate Solution and Naphthol AS-BI Phosphoric Acid Solution if reagents turn bright yellow or if precipitate forms.

Discard Pararosaniline Solution if solution does not turn amber upon addition of Sodium Nitrite Solution.

Sodium Nitrite Solution, Phosphate Buffer Solution should be discarded if turbidity develops.

PREPARATION:

Warm α-Naphthyl Butyrate Solution and Naphthol AS-BI Phosphoric Acid Solution to 37°C.

Sodium Nitrite Tablet Solution, 4 g/dl is prepared by dissolving 1 tablet in 6.25 ml deionized water. Store this solution tightly stoppered at 2–8°C. Warm to room temperature before use. Discard if turbidity develops.

Phosphate Buffer Solution is prepared by dissolving contents of Phosphate Buffer in 500 ml deionized water. Buffer has concentration of 0.067 mol/l, pH 7.7 at 25°C. Store in refrigerator (2–8°C). Discard if microbial growth is evident.

NOTE: Microbial growth may be retarded by filtration through a 0.22 micron Filter Unit.

Methylene Blue Counterstain is prepared by adding 5 ml Methylene Blue Solution to 45 ml deionized water. Mix well. Prepare fresh daily.

Citrate-Acetone-Formaldehyde Fixative: To 25 ml Citrate Solution add 65 ml Acetone and 8 ml of 37% Formaldehyde. Place in glass bottle and cap tightly. Store in refrigerator (2–8°C). Warm to 18–26°C prior to use. Stable if stored tightly capped in refrigerator

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.

Samples may be collected in either EDTA or heparin. After fixation slides may be stored at room temperature for at least 2 weeks. If mononuclear cells are to be isolated using HISTOPAQUE®-1077, separation should be performed within 4 hours although fair recovery has been noted after 24 hours.[25] Blood films, particularly from leukopenic individuals, are not recommended for these procedures since evaluation of this material is quite time consuming. Cytocentrifuge or buffy coat preparations should be employed. Bone marrow films and tissue touch preparations pose no problems with respect to microscopic evaluation.

SPECIALS MATERIALS REQUIRED BUT NOT PROVIDED:

Acetone, ACS Reagent
Formaldehyde, 37%
Water bath capable of maintaining 37°C shielded from light

NOTES:

Cells from healthy donors should be included with each test.

Cells isolated on polysucrose-sodium diatrizoate gradients may be stored in liquid nitrogen (LN_2) for control purposes. To accomplish this, 1 x 10^7 - 10^8 cells are frozen at 1 ± 0.3°C per minute in a medium containing 50% fetal calf serum, 40% RPMI-1640 (or other appropriate tissue culture fluids) and 10% dimethylsulfoxide (DMSO). They may be stored in either the liquid or vapor phase of LN_2.

Slides should be evaluated using 1000X magnification. Dydimium filters may enhance color particularly of the diffuse staining α-NB.

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:

ACID PHOSPHATASE PROCEDURE:

Reagents

Naphthol AS-BI Phosphoric Acid Solution
Fast Garnet GBC Base Solution
Sodium Nitrite Solution
Citrate Solution
Acetate Solution
Methylene Blue Solution

1. Prewarm enough deionized water for a day's use to 37°C.
2. Immediately prior to fixation, add 1 ml of Sodium Nitrite Solution to 1 ml Fast Garnet GBC Base Solution. Mix gently by inversion and let stand 2–5 minutes.
3. Add solution from Step 2 to 38 ml prewarmed deionized water.
4. Add 5 ml Acetate Solution.
5. Add 5 ml Naphthol AS-BI Phosphoric Acid Solution. Mix well and pour into Coplin jar. Solution will be amber. Formation of precipitate indicates reagent deterioration.
6. Fix slides for 30 seconds in Citrate-Acetone-Formaldehyde Fixative at room temperature (18–26°C). Rinse in deionized water for 45–60 seconds. Do not allow slides to dry.
7. Immediately after rinsing, place slides into solution from Step 5 and incubate 1 hour at 37°C.

NOTE: If slides are not placed in incubation solution after fixation, they must be allowed to air dry for at least 45 minutes.

8. After 1 hour remove slides from Coplin jar and rinse at least 2 minutes in running tap water. Discard staining solution.
9. Allow slides to air dry at least 15 minutes before counterstaining.
10. Counterstain 2 minutes in Methylene Blue Counterstain.
11. Rinse in deionized water.

α-NAPHTHYL BUTYRATE ESTERASE PROCEDURE:

Reagents

α-Naphthyl Butyrate Solution
Pararosaniline Solution
Sodium Nitrite Tablets
Phosphate Buffer
Methylene Blue Solution
Citrate Solution

1. Prewarm Phosphate Buffer Solution to 37°C.
2. Immediately prior to fixation, add 1.5 ml of Sodium Nitrite Tablet Solution to 1.5 ml Pararosaniline Solution. Mix gently by inversion and let stand at least 5 minutes, then add to 40 ml of prewarmed Phosphate Buffer Solution.
3. Add 5 ml α-Naphthyl Butyrate Solution.
4. Mix well and pour into Coplin jar. Solution will be amber. Formation of precipitate indicates reagent deterioration.
5. Fix slides for 10 seconds in Citrate-Acetone-Formaldehyde Fixative at room temperature (18–26°C). Rinse in deionized water for 45 seconds. Do not allow slides to dry.
6. Immediately after rinsing, place slides into solution form Step 4 and incubate 1 hour at 37°C. NOTE: If slides are not placed in incubation solution after fixation, they must be allowed to air dry for at least 45 minutes.
7. After 1 hour remove slides from Coplin jar and rinse 2–3 minutes in running tap water. Discard staining solution.
8. Allow slides to air dry at least 15 minutes before counterstaining.
9. Counterstain 5 minutes in Methylene Blue Counterstain.
10. Rinse in deionized water.

PERFORMANCE CHARACTERISTICS

ACID PHOSPHATASE: Focal (dot) staining of lymphocytes is suggestive of thymic lineage (T-cells). **α-NAPHTHYL BUTYRATE ESTERASE:** The focal (dot) staining pattern observed in some lymphocytes is associated with mature T-cells bearing Fc receptors for IgM. This subset overlaps the T-helper cell population to some extent but is not an accurate measure of helper function. The diffuse or granular staining lymphocytes bear receptors to the Fc portion of IgG and overlap to some extent the T suppressor cell population.

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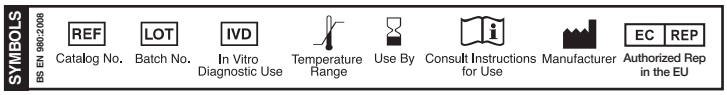

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