



# SIGMA-ALDRICH®

## LYMPHOCYTE ENZYMES

(Procedure No. 181)

### INTENDED USE

Sigma-Aldrich Lymphocyte Enzyme Kits are for use in the detections of acid phosphatase (Kit No. 181-A),  $\alpha$ -naphthyl butyrate esterase (Kit No. 181-B) or  $\beta$ -glucuronidase (Kit No. 181-C) 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.<sup>1-14</sup> 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),  $\beta$ -glucuronidase (BG), and  $\alpha$ -naphthyl butyrate esterase ( $\alpha$ -NB), display characteristic age-related cytochemical profiles. AcP is acquired by early fetal thymocytes and is retained throughout T-cell differentiation.<sup>15-16</sup> BG, appearing later in development, is found in postgestational thymocytes, mature circulating T-cells<sup>17</sup> and may be a true Pan T-enzyme.<sup>18-20</sup> An epitope of  $\alpha$ -NB, reactive over a narrow pH range (5.7-6.0) is expressed by mature T-cells and medullary thymocytes.<sup>18-20</sup> From their results, Basso et al.,<sup>16</sup> has proposed a maturation scheme whereby differentiating T-cells progress from AcP+, BG-,  $\alpha$ -NB- to AcP+, BG+,  $\alpha$ -NB- and finally AcP+, BG+,  $\alpha$ -NB+.

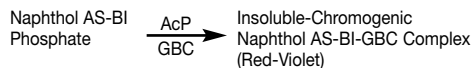
The majority of mature T-cells, as defined by sheep red blood cell rosetting, display a distinct focal (dot) pattern when stained for AcP, BG and  $\alpha$ -NB<sup>21,22</sup> and express surface receptors for IgM (T<sub>M</sub>, T<sub>H</sub>, Fc).<sup>23</sup> Those characterized by a diffuse/granular stain express Fc receptors for IgG (T<sub>G</sub>, gFc).<sup>23</sup> There is some evidence that the T<sub>M</sub>T<sub>G</sub> subsets may overlap the OKT-4/OKT-8 (helper-suppressor) subsets.<sup>17-23</sup> Discordant data concerning this relationship have been reported.<sup>23,24</sup> 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 three 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  $\alpha$ -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  $\alpha$ -Naphthyl Butyrate Esterase is less sensitive than the  $\alpha$ -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  $\alpha$ -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,  $\alpha$ -naphthyl butyrate esterase and  $\beta$ -glucuronidase are then visualized by the following simultaneous capture principles.

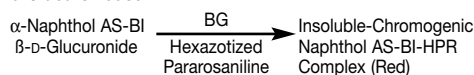
#### Acid Phosphatase:



#### $\alpha$ -Naphthyl Butyrate Esterase:



#### $\beta$ -Glucuronidase:



### REAGENTS

$\alpha$ -NAPHTHYL BUTYRATE SOLUTION, Catalog No. 180-1  
 $\alpha$ -Naphthyl Butyrate, 2.4 g/l, in methanol solution with solubilizers.

#### NAPHTHOL AS-BI PHOSPHORIC ACID SOLUTION,

Catalog No. 180-2  
Naphthol AS-BI phosphoric acid, 4 g/l, in methanol solution with solubilizers.

#### NAPHTHOL AS-BI $\beta$ -D-GLUCURONIC ACID SOLUTION,

Catalog No. 180-3  
Naphthol AS-BI  $\beta$ -D-glucuronic acid, 2.5 g/l, in methanol solution with solubilizers.

#### PARAROSANILINE SOLUTION, Catalog No. 180-4

Pararosaniline, 40 g/l, in 2 mol/l hydrochloric acid.

#### FAST GARNET GBC BASE SOLUTION, Catalog No. 387-2

Fast garnet GBC base, 7.0 mg/ml, in 0.4 mol/l hydrochloric acid and stabilizer.

#### SODIUM NITRITE SOLUTION, Catalog No. 91-4

Sodium nitrite, 0.1 mol/l.

#### SODIUM NITRITE TABLETS, Catalog No. 180-9

Sodium nitrite, 250 mg per tablet.

#### CITRATE SOLUTION, Catalog No. 91-5

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. 386-3

Acetate buffer, 2.5 mol/l, pH 5.2.

#### PHOSPHATE BUFFER, Catalog No. 180-5

Sodium and potassium phosphates.

#### METHYLENE BLUE SOLUTION, Catalog No. 180-8

Methylene blue, 1.4% (w/v) in 95% ethanol.

#### STORAGE AND STABILITY:

Store  $\alpha$ -Naphthyl Butyrate Solution, Naphthol AS-BI Phosphoric Acid Solution and Naphthol AS-BI  $\beta$ -D-Glucuronic 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  $\alpha$ -Naphthyl Butyrate Solution, Naphthol AS-BI Phosphoric Acid Solution and Naphthol AS-BI  $\beta$ -D-Glucuronic Acid Solution if reagents turn 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  $\alpha$ -Naphthyl Butyrate Solution, Naphthol AS-BI Phosphoric Acid Solution and Naphthol AS-BI  $\beta$ -D-Glucuronic 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, Catalog No. 180-5, 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, Catalog No. 180-8, to 45 ml deionized water. Mix well. Prepare fresh daily.

Citrate-Acetone-Formaldehyde Fixative: To 25 ml Citrate Solution, Catalog No. 91-5, 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 23-26°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 for any updated risk, hazard or safety information.

#### US Risks and Safety Statements

$\alpha$ -Naphthyl Butyrate Solution is FLAMMABLE and TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. Take precautionary measures against static discharges. Avoid contact with skin. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Naphthol AS-BI Phosphoric Acid Solution is FLAMMABLE and TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Naphthol AS-BI  $\beta$ -D-Glucuronic Acid Solution is FLAMMABLE and TOXIC. Harmful by inhalation and if swallowed. Irritating to eyes and skin. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Pararosaniline Solution is TOXIC. Toxic by inhalation. Causes burns. May cause cancer. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Target organs: Liver and thyroid. California Prop 65 carcinogen.

Avoid Contact and Inhalation of Phosphate Buffer.

Methylene Blue Solution is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Sodium Nitrite Tablets are TOXIC, OXIDIZING and dangerous to the environment. Contact with combustible material may cause fire. Toxic if swallowed. Very toxic to aquatic organisms. Keep away from combustible material. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid release to the environment. Refer to special instructions/safety data sheets.

Fast Garnet GBC Base Solution is TOXIC. Harmful if swallowed. Toxic by inhalation. Causes burns. May cause cancer. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Target organs: Liver and kidneys.

Acetate Solution - Caution: Substance not yet fully tested.

Acetone is FLAMMABLE and an IRRITANT. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Target organs: Liver and kidneys.

Formaldehyde solution is TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Causes burns. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. May cause heritable genetic damage. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Use only in well ventilated areas.

EU Risks and Safety Statements (Caution: Substances not yet fully tested)

$\alpha$ -Naphthyl Butyrate Solution is TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Naphthol AS-BI Phosphoric Acid Solution is TOXIC. Flammable. Toxic by inhalation, in contact with skin and if swallowed. Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Naphthol AS-BI  $\beta$ -D-Glucuronic Acid Solution is TOXIC. Flammable. Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Pararosaniline Solution is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.

Phosphate Buffer: Caution: Substance not yet fully tested.

Methylene Blue Solution is HIGHLY FLAMMABLE. Highly flammable. Keep container tightly closed. Keep away from sources of ignition - no smoking.

Sodium Nitrite Tablets are OXIDIZING, TOXIC and dangerous to the environment. Contact with combustible material may cause fire. Toxic if swallowed. Very toxic to aquatic organisms. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Avoid release to the environment. Refer to special instructions/safety data sheets.

Fast Garnet GBC Base Solution is TOXIC. Harmful if swallowed. May cause cancer. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Acetate Solution - Caution: Substance not yet fully tested. Acetone is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Formaldehyde solution is TOXIC. Toxic by inhalation, in contact with skin and if swallowed. Causes burns. Limited evidence of a carcinogenic effect. May cause sensitization by skin contact. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Use only in well ventilated areas.

## PROCEDURE

### SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. 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<sup>®</sup>-1077, separation should be performed within 4 hours although fair recovery has been noted after 24 hours.<sup>25</sup> 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

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<sub>2</sub>) for control purposes. To accomplish this, 1 x 10<sup>7</sup> - 10<sup>9</sup> 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<sub>2</sub>.

Slides should be evaluated using 1000X magnification. Didymium 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, Catalog No. 180-2

Fast Garnet GBC Base Solution, Catalog No. 387-2

Sodium Nitrite Solution, Catalog No. 91-4

Citrate Solution, Catalog No. 91-5

Acetate Solution, Catalog No. 386-3

Methylene Blue Solution, Catalog No. 180-8

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, Catalog No. 91-4, to 1 ml Fast Garnet GBC Base Solution, Catalog No. 387-2. 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, Catalog No. 386-3.
5. Add 5 ml Naphthol AS-BI Phosphoric Acid Solution, Catalog No. 180-2. 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 (23-26°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, Catalog No. 180-1

Pararosaniline Solution, Catalog No. 180-4

Sodium Nitrite Tablets, Catalog No. 180-9

Phosphate Buffer, Catalog No. 180-5

Methylene Blue Solution, Catalog No. 180-8

Citrate Solution, Catalog No. 91-5

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, Catalog No. 180-4. 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, Catalog No. 180-1.
  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 (23-26°C). Rinse in deionized water for 45 seconds. Do not allow slides to dry.
  6. Immediately after rinsing, place slides into solution from 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.

#### β-GLUCURONIDASE PROCEDURE:

##### Reagents

Naphthol AS-BI β-D-Glucuronic Acid, Catalog No. 180-3

Pararosaniline Solution, Catalog No. 180-4

Sodium Nitrite Tablets, Catalog No. 180-9

Acetate Solution, Catalog No. 386-3

Methylene Blue Solution, Catalog No. 180-8

Citrate Solution, Catalog No. 91-5

1. Prewarm enough deionized water for a day's use to 37°C.
  2. Immediately prior to fixation, add 0.5 ml of Sodium Nitrite Tablet Solution to 0.5 ml Pararosaniline Solution, Catalog No. 180-4. Mix gently by inversion and let stand 5 minutes, then add to 38 ml of prewarmed deionized water.
  3. Add 5 ml Acetate Solution, Catalog No. 386-3.
  4. Add 5 ml Naphthol AS-BI β-D-Glucuronic Acid Solution, Catalog No. 180-3.
  5. 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 (23-26°C). Rinse in deionized water for 45 seconds. Do not allow slides to dry.
  7. Immediately after rinsing, place slides into solution from Step 5 and incubate 90 minutes at 37°C protected from light.
- 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 90 minutes remove slides from Coplin jar and rinse 2-3 minutes in running tap water. Discard staining solution.
  9. Allow slides to air dry at least 15 minutes before counterstaining.
  10. Counterstain 3 minutes in Methylene Blue Counterstain.
  11. 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 for the Fc portion of IgG and overlap to some extent the T suppressor cell population.

**β-GLUCURONIDASE:** Positive staining appears associated with mature thymocytes, circulating T-cells and a subpopulation of immature B-cells. The focal (dot) and diffuse-granular patterns mark in a fashion analogous to α-naphthyl butyrate esterase. Monocytes and granulocytes are usually negative.

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

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