

Cytochemical demonstration of non-specific leukocyte esterase.

### PRINCIPLE OF TEST

Cellular esterase are accepted to be a series of different enzymes acting upon select substrates. The 'specific' esterase of granulocytes can be demonstrated using the substrate Naphthol AS-D Chloroacetate. The 'non-specific' esterase of monocytes can be demonstrated using a-Naphthyl Acetate. The demonstration of 'non-specific' esterase is achieved by incubating fixed blood, bone marrow a tissue touch preparations in a solution of a-Naphthyl Acetate and the Diazonium salt of pararosaniline. Enzymatic cleavage of the esterase releases the Naphthol which couples with the Diazonium salt to form coloured deposits at site of ANAE activity.

### REAGENTS

- |                                  |  |                                      |
|----------------------------------|--|--------------------------------------|
| 1 - a-Naphthyl Acetate caps x 10 | 2 - Pararosaniline reagent 2 x 25ml    | 3 - Sodium nitrite reagent 1 x 30ml  |
| 4 - Citrate solution 4 x 25ml    | 5 - Haematoxylin Counterstain 1 x 50ml | 6 - Sodium Fluoride reagent 1 x 20ml |
| 7 - Methanol 3 x 25ml            | 8 - Tris-maleate concentrate 3 x 35ml  |                                      |

Reagent components in this kit are for 'In Vitro Diagnostic use only'. Use this product in a well ventilated place or in a fume hood. Wear skin & eye protection when in use. Standard precautions in handling laboratory reagent should be followed. Refer to Material Safety Data section.

### STORAGE AND STABILITY

Store kit components in the kit box provided in a refrigerator. Test reagents at Retest date and if still performing satisfactory continue to use for 3 months further setting a new Retest date for the end of this 3 month period and repeat this process until deterioration of reagents is detected in test results then discard the remaining reagents and replace the kit.

Tris-Maleate concentrate and Citrate solution are suitable for use only in the absence of microbial growth, although inhibitors are used if microbial growth appears before expiry date please contact us for free replacements.

### ADDITIONAL REAGENTS REQUIRED

Acetone, Reagent Grade.

Formaldehyde 37%, Reagent Grade.

### SPECIMEN COLLECTION AND STORAGE

Blood, bone marrow or tissue touch preparations may be used. Samples using Heparin or EDTA anticoagulants are acceptable. Frozen tissue sections can be used successfully.

Blood and bone marrow samples may be stored fixed at room temperature (18-25DC) for 6 weeks or unfixed for up to 1 week without appreciable loss of enzyme activity.

### REAGENT PREPARATION AND SETUP

Prepare smears or sections.

Prepare the following solutions:

- 1 - CAF Fixative.  
To 25ml Citrate solution add 65ml Acetone and 8ml 37% Formaldehyde. Mix well. Ensure CAF Fixative is at room temperature.
- 2 - To a glass tube or beaker add 3ml Pararosaniline reagent and 1ml Sodium nitrite reagent. Mix and keep at room temperature for approx 2 minutes. Oxides of Nitrogen are produced – work in a fume hood, wear appropriate face mask.
- 3 - In a Coplin Jar or similar add:
  - (a) a-Naphthyl acetate contents of 1 capsule. Rinse out the capsule with 8ml Methanol into the Coplin Jar. Ensure the crystals are fully dissolved before proceeding.
  - (b) Content of beaker prepared in step 2.
  - (c) 30ml prewarmed deionised water use a little of the water to rinse beaker and add to Coplin Jar.
  - (d) 10ml Tris-Maleate concentrate.
  - (e) Mix well maintain temperature at 37DC.

### TECHNIQUE (ANAE)

- 1 - Fix slides in CAF Fixative for 30 seconds. Agitate steadily.
- 2 - Rinse slides thoroughly in running deionised water for 45-60 seconds. Do not allow slides to dry.
- 3 - Place immediately in the incubation mixture and incubate for 30 minutes at 37DC protected from light.
- 4 - After the incubation period is complete. Rinse slides in running tap water.
- 5 - If required - immerse in counterstain for 1 – 2 minutes ( this step can be omitted if required).
- 6 - Rinse briefly in TAPWATER and air dry.
- 7 - Mount in aqueous media if required.
- 8 - Examine microscopically.

### FLUORIDE INHIBITION PROCEDURE

ANAE is found primarily in cells of monocytic lineage in this procedure. This reaction in monocytes is Sodium Fluoride sensitive. It should be noted that megakaryocytes and erythroid precursors also show positive ANAE reaction. Lymphocytes and mature granulocytes may show positive ANAE reaction in this test. To differentiate these cells from monocytes the Sodium Fluoride inhibition procedure should be used. The monocyte enzyme is inactivated in the presence of Naf.

### TECHNIQUE (Naf)

Proceed exactly as described in the 'reagent preparation and set up' section above except prepare 2 separate incubating mixtures. To one of the Coplin Jars containing the incubation mixture add 1ml of Sodium Fluoride solution and mix well. Label the jars appropriately. Carry out the test following the technique for ANAE above with the Naf inhibited test running simultaneously alongside the normal ANAE test.

## RESULTS

- 1 - ANAE  
Monocytes should show red/brown profuse granulation. Lymphocytes also show enzyme activity. Red dot like reactions occasionally seen in Lymphocytes. Nuclei stain shades of blue.
- 2 - ANAE-Naf INHIBITED  
A negative result will be obtained for all cells of monocytic lineage.

## SCORING

Select an area of film with few erythrocytes. Sites of ANAE activity will appear as red/brown granulation. Score from 0 to 4+ based on the intensity of precipitated dye in the cytoplasm of each cell type. An example of a scoring format is given below.

SCORE	INTENSITY OF STAINING
0	Absent
1+	Faint-moderate
2+	Moderate-strong
3+	Strong
4+	Very strong

Typical cellular ANAE activities is illustrated in the table below:

CELL	ANAE REACTION
Myeloblasts	+
Promyleocytes	+
Neutrophils	--
Eosinophils	--
Basophils	--
Monocytes	+
Lymphocytes	+
Lymphoblasts	+
Megakaryocytes	+
Erythroblasts	+
Plasma cells	+
Mast cells	--
Hairy cells	+
Histocytes	+

## LIMITATIONS

All glassware must be thoroughly rinsed with deionised water. Traces of detergent can interfere with enzyme systems. Thoroughly rinse slides after fixation in deionised water. Traces of Formaldehyde can interfere with enzyme systems. Temperature of reaction must be at 37°C. Used glass Coplin Jars in preference to plastic ware. This procedure depends on the subjective of staining cells. Laboratories should establish their own normal ranges.

## MATERIAL SAFETY DATA SECTION

Use these kit reagents in a well ventilated place or in a fume hood as vapours of OXIDES OF NITROGEN, such as Nitric oxide and Nitrogen dioxide are likely to be produced although in small quantity. These vapours are harmful/dangerous if inhaled therefore efficient ventilation / fume hood should be used. Reagents in this kit are irritating to eyes and skin. Do not consume these reagents. Wear suitable protective clothing i.e. skin, eyes and face protection (including face mask to stop organic vapours ( Oxides of Nitrogen). P301 If SWALLOWED- rinse mouth with water, seek emergency medical attention. P304 - If INHALED- move to clear air zone. P302/305 - If on SKIN or in EYES- wash with soap and water/apply eye bath. P314 - get medical attention if you feel unwell and show this sheet.

- 1 - a-Naphthyl acetate powder is an irritant to eyes, skin and respiratory system.
- 2 - Pararosaniline reagent - May cause irritation/burns to skin, eyes and internal organs. Treat as for hydrochloric acid contamination. Will cause red/purple marks to skin (wash with soap & water several times)
- 3 - Sodium nitrite reagent - Toxic by inhalation and ingestion. Oxidising agent. May cause eye and skin irritation. Forms oxides of Nitrogen in contact with acidic compounds such as the Pararosaniline reagent in this kit.
- 4 - Citrate solution - No special hazard with this reagent.
- 5 - Counterstain - May cause irritation to eyes and stains to the skin. Wash with soap & water if stains to the appear.
- 6 - Sodium fluoride reagent - Toxic if taken internally and possibly by skin absorption. May cause irritation to eyes.
- 7 - Methanol – Highly flammable liquid and vapour. Keep away from sources of ignition. Toxic by inhalation and ingestion.
- 8 - Tris maleate concentrate - Irritant to skin, eyes and internal organs.

H: 319+331+301+225  
P: 210+281+284

For more complete information on Health & Safety, Fire fighting, Storage, Transport etc please see MSDS for this product.

## ACCIDENTAL SPILLAGE & WASTE DISPOSAL

In the volumes supplied in the kit and when in use, this product is unlikely to present a serious spillage risk. However, the following information is provided to deal with any spillage or disposal problem that may arise.

a-naphthyl acetate capsules – mop up spillage with damp cloth, rinse cloth under tapwater diluting to public sewer.

Pararosaniline reagent - same treatment as a-naphthyl acetate.

Sodium nitrite reagent – this is a dilute solution and in the quantity provided offers little environmental risk. Mop up spillage with cloth, rinsing cloth under tapwater diluting to public sewer.

Citrate solution – same treatment as a-naphthyl acetate

Counterstain – same treatment as a-naphthyl acetate.

Sodium fluoride – this is a dilute reagent and in the quantity provided offers little environmental risk. Mop up spillage with cloth , rinsing cloth under tapwater diluting to public sewer.

Methanol – same treatment as a-naphthyl acetate.

Tris-maleate buffer – same treatment as a-naphthyl acetate.

## UNSATISFACTORY PERFORMANCE

As part of our duty to monitor product performance and our policy of continual improvement. Please report to us any unsatisfactory performance you may experience with this product. If any reagent degrades before expiry date of shelf life we will replace that reagent free of charge.

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product and that the product conforms to the information contained in this leaflet.

The user should however, determine the suitability of this product for their particular use.

If you wish to report any findings to us or if you require help or further information on the use of this product please contact us.

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