

USE

Cytochemical demonstration of specific and 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 either esterase is achieved by incubating fixed blood, bone marrow or tissue touch preparations in a solution of the substrate and a Diazonium salt. Esterase enzymatic cleavage releases the Naphthol which couples with the Diazonium salt to form coloured deposits at site of enzyme activity.

REAGENTS

- | | |
|--|---|
| 1 - a-Naphthyl Acetate Capsules (ANAE) 10 capsules | 7 - Substrate & coupling compound (NCAE) 10 capsules each |
| 2 - Pararosaniline reagent 2 x 25ml | 8 - Tris-maleate concentrate pH 6.30 50ml |
| 3 - Sodium nitrite reagent 25ml | 9 - Tris-maleate concentrate pH 7.60 100ml |
| 4 - Citrate solution 2 x 100ml | 10 - DMF Stabiliser 30ml |
| 5 - Sodium fluoride reagent 20ml | 11 - Counterstain methyl green 50ml |
| 6 - Methanol 100ml | |

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 all kit capsule reagents at -5 to -20 C. Store other kit components kit components refrigerated or at cool room temperature in a darkened cupboard. Note expiry date on the pack.

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.

Storage of some components in a refrigerator (Tris maleate concentrates) may cause crystallisation. If this happens warm the reagent bottle in a beaker of hot water until the crystallised component re-dissolves.

Haematoxylin counterstain - keep lid on tight as this product will decompose more rapidly when exposed to air. Discard if solution turns brown.

Store CAF Fixative in refrigerator when not in use.

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:

STAGE 1 (ANAE)

- 1 - CAF Fixative.
To 25ml Citrate solution add 65ml Acetone and 8ml 37% Formaldehyde. Mix well. Ensure CAF Fixative is at room Temperature. Store in dark bottle at room temperature. Usable for 1 month.
- 2 - To a glass tube or beaker add 3ml Pararosaniline reagent and 1 ml Sodium nitrite reagent. Mix and keep at room temperature for approx 1 minute.
- 3 - In a Coplin Jar or similar add:
 - (a) a-Naphthyl acetate contents of 1 capsule. Rinse out the capsule with 8-10ml Methanol into the jar, ensure jar contents dissolved before proceeding.
 - (b) Content of beaker prepared in step 2.
 - (c) 30ml prewarmed (37 C) deionised water use a little of the water to rinse beaker and add to Coplin Jar.
 - (d) 10ml Tris-Maleate concentrate pH 7.60
 - (e) Mix well maintain temperature at 37°C.
pH should be between 5.80-6.50

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. Traces of formalin can affect reaction. Do not allow slides to dry.
- 3 - Place immediately in the incubation mixture and incubate for 10 minutes at 37DC protected from light.
- 4 - After the incubation period is complete the slide should be a light brown colour.
- 5 - Rinse for **5 minutes** in a beaker of running tap water. This is necessary to clear the background to prove a satisfactory contrast. The smear should now be a pale or very pale brown colour

Proceed to STAGE 2 or continue to finish if this ANAE stage only is required.

- 5 - Place slide in Counterstain for 1 minute followed by short 5second rinse in DI water.

- 5 - Mount in aqueous media if required. (CAT: M400).
- 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 green.
- 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	+

STAGE 2 (NCAE)

Incubation mixture - To a Coplin Jar or similar,

ADD IN THE FOLLOWING ORDER:

- a) Contents of **one capsule each** of substrate (Naphthol ASD Chloroacetate) and coupling compound.
- b) 3ml of DMF stabiliser using a few drops to rinse out the capsule into the Coplin Jar.
- c) Ensure the compound has dissolved in the DMF stabiliser before proceeding.
- d) 40ml of pre-warmed deionised water (37DC) and mix well [keep at 37-38DC]
- e) 5ml Tris-Maleate concentrate pH 6.30-6.40

TECHNIQUE (NCAE)

- 1 - Using the slides from the ANAE reaction after the 5 minute rinse in tap water, rinse again briefly in deionised water.
- 2 - Place immediately in the incubation mixture and incubate for 20 minutes at 37 DC protected from light.
- 3 - After the incubation period is complete. Rinse slides in a beaker of running tap water for **5 minutes**.
- 4 - Counterstain in Methyl green for 1 minute.
- 5 - Rinse in Deionised water for 20-30 seconds.
- 6 - Rinse in tap water for 30 seconds and air dry.
- 7 - Examine under oil or
- 8 - Mount in aqueous media if required. (CAT: M400)
- 9 - And examine microscopically.

RESULTS

- 1 - NCAE
This enzyme is accepted as specific for cells of granulocytic lineage. Sites of enzymatic activity should show dark blue or gray-black granulation/diffuse granulation. Reaction is weak or not seen in monocytes and lymphocytes.

SCORING

Select an area of film with few erythrocytes. Sites of NCAE activity will appear as gray-black 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 NCAE activities is illustrated in the table below:

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

LIMITATIONS & GUIDANCE

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 DC. Use glass Coplin Jars in preference to plastic ware. This procedure depends on the subjective rating of staining cells. Laboratories should establish their own normal ranges. Staining can be intense in neutrophils. To produce a clearer stain picture incubation time can be reduced if demonstration if granulation is required. Thickness of smear can alter stain picture obtained, thin smears preferred.

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). In all cases take off contaminated clothing and wash with plenty of water. In case of contact with skin and eyes, rinse with large volumes of water or treatment from an eye bath station for 5 minutes and seek medical attention if soreness persists. If feeling unwell seek immediate medical attention and show this sheet. If any substance is taken internally rinse out mouth with plenty of water and seek immediate medical advise on what treatment to administer 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 - Methyl green reagent 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 irritate skin & eyes.
- 7 - Methanol - Highly Flammable- Toxic if taken internally (Tetrogen) and by skin absorption. Irritant to eyes, skin and internal organs.
- 8 - Tris maleate pH 7.60 concentrate - Irritant to skin, eyes and internal organs.
- 9 - Substrate and coupling compound (mixture of Fast Red Violet LB salt and Naphthol AS-D chloroacetate) Fast red violet is an azo-dye and is toxic if taken internally or by breathing dust. Will irritate eyes and internal organs. Naphthol AS-D chloroacetate powder may irritate eyes skin and internal organs. Do not breath dust. Wear face mask when using
- 10 - Citrate solution - No special hazard with this reagent.
- 11 - Tris maleate pH6.30 concentrate. Irritant to skin eyes and internal organs.

12 - Haematoxylin solution - Maybe harmful if taken internally. Will discolour skin. Will irritate eyes, skin and internal organs. Contains aluminium salts and haematoxylin dye.

13 - DMF stabiliser - contains solubilisers & dimethylformamide. FLAMMABLE. Harmful by inhalation. Do not breath vapour. May cause irritation to eyes, skin and internal organs. Danger of irreversible effects. Maybe absorbed through skin. May cause harm to the unborn child. Wear appropriate protection when in use.

R: 10-20-21-22-23-25-36-37-38-61 S: 16-20-21-24-25-36-39

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

Methyl green 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.

2 – Methoxyethanol – same treatment as a-naphthyl acetate.

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

Substrate & coupling compound – mop up spillage with damp cloth, rinsing cloth under tap water diluting to public sewer.

Tris-maleate concentrate – same treatment as for Substrate & coupling compound.

Citrate solution - same treatment as for Substrate & coupling compound

Haematoxylin Gill No 3 - same treatment as for Substrate & coupling compound

DMF Stabiliser –Consult local regulations . In the quantities supplied and used in the test, it is unlikely to pose a significant danger to the environment - same treatment as for Substrate & coupling compound. However, local water authorities may be consulted about local regulations for the release of this substance to the environment (bearing in mind only 2ml is used in each test). If local regulations prohibit its release then any spillage must be absorbed into paper or suitable absorbent and any spent incubation mixture kept in a suitable container for disposal by a licensed waste disposal contractor.

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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