

GCC NAPHTHOL AS-D CHLOROACETATE ESTERASE SP930 (SPECIFIC ESTERASE)

USE

Cytochemical demonstration of specific leukocyte esterase.

PRINCIPLE OF TEST

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

REAGENTS

1 -Naphthol ASD Chloroacetate 10 capsules 2 – Fast Red Violet 10 capsules 3 - Tris-Maleate concentrate 50 ml
3 -Citrate solution 2 x 100ml 4 -Haematoxylin solution. Gill No. 3 50 ml 5 -DMF stabiliser 25ml

Reagent components in this kit are for 'In Vitro Diagnostic use only '. Wear skin & eye protection when in use. Standard precautions in handling laboratory reagents should be followed. Refer to Material Safety Data section before use.

STORAGE AND STABILITY

Store Naphthol ASD Chloroacetate capsules at -5 to -20 C . Store other kit reagents in a refrigerator or at cool temperature in a darkened cupboard. 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 a free replacement.

Haematoxylin - 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 (18-25 DC) for 6 weeks or unfixed for up to 1 week without appreciable loss of enzyme activity.

REAGENT PREPARATION AND SET UP

Prepare smears or sections.

Prepare the following solutions:

1 -CAF Fixative.

To 25ml Citrate solutions add 65ml Acetone and 8ml 37% Formaldehyde. Mix well. Ensure CAF Fixative is at room temperature.

2 -Incubation mixture - To a Coplin Jar or similar,

ADD IN THE FOLLOWING ORDER:

- Contents of one capsule each of substrate Naphthol ASD Chloroacetate and coupling compound Fast Red Violet.
- 2ml of DMF stabiliser using a few drops to rinse out each of the capsules into the Coplin Jar.
- Ensure the substrate and coupling compound have dissolved in the DMF stabiliser before proceeding.
- 40ml of pre-warmed deionised water (37DC) and mix well [keep at 37-38DC]
- 5ml Tris-Maleate concentrate pH 6.30-6.40

TECHNIQUE (NCAE)

- Fix slides in CAF Fixative for 30 seconds. Agitate steadily.
- Rinse slides thoroughly in running deionised water for 45-60 seconds or at least 3 changes of water. Do not allow slides to dry.
- Place immediately in the incubation mixture and incubate for 10-12 minutes at 37 DC protected from light.
- After the incubation period is complete. Rinse slides for 5 MINUTES in running deionised water.
- Counterstain in Haematoxylin Gill No. 3 for 3 minutes.
- Rinse in tap water for 30 seconds and air dry.
- Examine under oil or
- Mount in aqueous media if required.
- And examine microscopically.

RESULTS

1 -NCAE

This enzyme is accepted as specific for cells of granulocytic lineage. Sites of enzymatic activity should show bright red 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 bright red 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 of granulation is required.

MATERIAL SAFETY DATA SECTION.

Reagents in this kit are irritating to eyes and sensitive skin. The DMF stabiliser is **FLAMMABLE**. Keep away from sources of ignition. Do not consume the reagents. Wear suitable protective clothing i.e. skin, eyes and face protection. Wear appropriate dust / vapour mask. 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 several minutes and seek medical attention. Show this sheet.

If any substance is taken internally rinse out mouth with plenty of water and seek medical advise on what treatment to administer. Show this sheet.

- 1 - Coupling compound Fast red violet is an azo-dye and is toxic if taken internally or by breathing dust. Will irritate eyes and internal organs. Substrate Naphthol AS-D chloroacetate powder may irritate eyes skin and internal organs. Do not breathe dust. Wear face mask when using.
- 2 - Tris maleate concentrate. Irritant to skin eyes and internal organs.
- 3 - Citrate solution - No special hazard with this reagent.
- 4 - Haematoxylin solution - Maybe harmful if taken internally. Will discolour skin. Will irritate eyes, skin and internal organs. Contains aluminium salts and haematoxylin dye.
- 5 - 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: 10-21-22-61 S:16-24-25

ACCIDENTAL SPILLAGE & WASTE DISPOSAL

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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