

USE

Cytochemical demonstration of myeloperoxidase activity for polymorphonuclearleukocytes in blood or bone marrow smears.

PRINCIPLE OF TEST

Blood or bone marrow films are incubated in a mixture of buffered Diaminobenzidine (DAB) and Hydrogen Peroxide. The brown reaction produced first formed is then enhanced with the Copper (II) Nitrate reagent followed by Haematoxylin and EA staining to give intense grey-black granulation at sites of neutrophil and monocyte myeloperoxidase activity.

REAGENTS

- 1 -Diaminobenzidine x 10 capsules
- 2 -Copper (II) Nitrate x 2 vials
- 3 -Tris-HCL Buffer Concentrate 1 x 50ml
- 4 -Glutaldehyde Solution, Buffered 4 x 30ml
- 5 - Haematoxylin Solution, Gill No. 3. 1 x 100ml
- 6 -EA Counterstain 3 x 30ml
- 7 - Scotts Tap Water Substitute Concentrate 1 x 100ml

Reagent components in this kit are for "IN-Vitro Diagnostic use only". Wear full body protection when using these reagents. Use them in a ventilated hood Standard precautions in the handling of laboratory reagents should be followed. Refer to Material Safety Data Section before use.

STORAGE AND STABILITY

Store reagents in the kit box provided at cool room temperature in a darkened cupboard in a safe place.
Store Copper (II) Nitrate solution (after reconstitution) at room temperature. Discard if turbidity develops.
Tris-HCL solutions and Scott Tap Water substitute - Discard if microbial growth or turbidity develops.
Store Glutaraldehyde-Acetone fixative in sealed glass bottles in refrigerator. Stable 2-3 months from preparation.
Haematoxylin - Discard if solution turns brown.

ADDITIONAL REAGENTS REQUIRED

- 1 -Hydrogen Peroxide 1%. 2 -Acetone, Reagent Grade. 3 -Ethanol 95% & 100%. 4 -Xylene.

SPECIMEN COLLECTION & STORAGE

Peripheral blood, buffy coat or bone marrow preparations may be used. Samples using heparin or EDTA anticoagulants are acceptable. Un-fixed samples may be stored in the dark at room temperature for several months without loss of activity.

Fixed samples may be kept at 4 DC for 1 week before staining.

REAGENT PREPARATION & SET UP

Prepare blood or bone marrow and allow to air dry.

Prepare the following solutions:

- 1 -Fixative solution-add 10ml Acetone to (1 bottle) 30ml Glutaraldehyde solution (store in refrigerator).
- 2 -TRIS-HCL working solution -dilute 1 volume Tris-HCL Concentrate with 9 volumes Deionised Water.
- 3 -Scotts Tap Water substitute-dilute 1 volume Concentrate with 9 volumes Deionised Water.
- 4 -Diaminobenzidine Solution-dissolve contents of 1 capsule in 50ml Tris-HCL working solution.
- 5 -Copper (II) Nitrate Solution-dissolve contents of 1 vial in 250ml deionised water. (store at room temperature).

Set up 12 staining jars or beakers containing the following:

- 1 -Glutaraldehyde-Acetone fixative at 5-10 DC.
- 2 -Diaminobenzidine solution.
- 3 -Copper (II) Nitrate solution.
- 4 -Haematoxylin solution Gill No. 3.
- 5 -Scotts Tap Water substitute working solution.
- 6 -EA Counterstain.
- 7 & 8-2 Jars 95% Ethanol.
- 9 & 10-2 Jars 100% (Absolute) Ethanol.
- 11 & 12-2 Jars Xylene.

TECHNIQUE

- 1 -Immediately before fixing slides add 1.0ml 1% Hydrogen Peroxide to Diaminobenzidine solution and mix well.
- 2 -Fix slides in Glutaraldehyde-Acetone fixative at 5-10 DC for 1 minute.
- 3 -Rinse for 10-15 seconds in deionised water.
- 4 -Incubate in Diaminobenzidine/Peroxide solution for 1 minute.
- 5 -Rinse for 10-15 seconds in deionised water.
- 6 -Immerse slides in Copper (II) Nitrate solution for 2 minutes. Asitate occasionally.
- 7 -Rinse for 10-15 seconds in deionised water.
- 8 -Immerse slide in Haematoxylin solution (Gills No.3) for 10 seconds or 10 dips.
- 9 -Rinse in 2 changes of deionised water for 5 seconds each rinse.

- 10 -Immerse slide in Scotts Tap water substitute working solution for 10-15 seconds (10-15 dips).
- 11 -Rinse for 10-15 seconds in deionised water.
- 12 -Flood slide with EA counterstain for 30 seconds.
- 13 -Rinse in 2 changes 95% Ethanol 3-5 seconds each.
- 14 -Rinse in 2 changes Absolute Ethanol 3-5 seconds each.
- 15 -Rinse in 2 changes Xylene 3-5 seconds each.
- 16 -Mount using DPX or similar mountant and examine.

NOTE

For those preferring a sorter method. Steps 12-15 may be eliminated resulting in Eosinophil peroxidase sites staining darker brown rather than red-orange.

RESULTS

Neutrophils and their precursors show grey-black granulation.
Developing granulocytes always give positive reactions.
Strong reaction is seen in promyelocytes and myelocytes but may be negative in early myeloblasts.
Less strong reaction is seen in promonocytes and monocytes.
Eosinophils stain red-orange or dark brown Basophils stain blue.
Monoblasts, lymphocytes and lymphoblasts fail to react.

SIGNIFICANCE

The FAB classification lists 7 subgroups of acute nonlymphocytic leukemia.

M1 5-15% of blasts may be positive.

M2 Positive.

M3 Positive.

M4 Positive (usually a mixed population of cells is noted).

M5a May be positive

M5b A fine granular deposit may be observed in more mature cells.

M6 Myelocytic cells are positive.

M7 Myelocytic cells are positive.

The results obtained should read in accordance with the FAB classification system.

A negative peroxidase reaction should not be considered pathognomic of acute nonmyelocytic leukemia.

A full cytochemical profile should be obtained from reactions such as x-Naphthyl Acetate Esterase, Naphthol-AS-D-Chloroacetate Esterase and Sudan Black B etc and use in conjunction with a clinical history before any diagnostic implications are made.

MATERIAL SAFETY DATA SECTION

Reagents in this kit are TOXIC or VERY TOXIC by inhalation or ingestion of dust or vapour or in contact with skin and eyes. The EA Counterstain is HIGHLY FLAMMABLE. The Copper II Nitrate is an OXIDISING AGENT – may cause fire if in contact with combustible materials. The Diaminobenzidine and Buffered Glutaraldehyde are VERY TOXIC by inhalation, ingestion and skin absorption. Danger of cumulative effects. 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. Work in a well ventilated place or under a fume hood. In all cases take off contaminated clothing and wash with plenty of soap & 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 advice on what treatment to administer. Show this sheet.

- 1 - Diaminobenzidine – pure powder form in capsules. Very Toxic by inhalation, ingestion or by skin contact. Wear full protective gear when in use. Face & dust mask. Use under ventilated hood.
- 2 - Copper II nitrate (pure powder form). Irritant to eyes and skin. See above.
- 3 - Buffered Glutaraldehyde reagent – this contains Glutaraldehyde <5% with harmless inorganic buffering salts. In aqueous solution. This reagent is TOXIC by ingestion, inhalation and skin absorption. Wear appropriate protection when in use. Work in fume hood. In case of accident follow above instructions. Show this sheet.
- 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 - Tris-HCL buffer conc. (Tris hydroxymethyl methylamine hydrochloride in aqueous solution). May irritate eyes & skin and internal organs.
- 6 - EA Counterstain – organic dyes in alcoholic solution.. Highly flammable. May irritate eyes and skin. Will stain skin. Wash with soap & water to remove stains.
- 7 - Scotts tap water substitute – Harmless inorganic salts in aqueous solution. No real risk in normal use. Concentrated solution may irritate eyes. If in contact wash with eye batch.

R: 8- 11-23-24-25-36-37-38-39-40 S: 2-7-16-23-24-25-26-36-37-39-51-61

ACCIDENTAL SPILLAGE & WASTE DISPOSAL

Diaminobenzidine - Consult local regulations . In the quantities supplied and used in the test, it is unlikely to pose a significant danger to the environment – therefore dispose of small test quantities to the public sewer diluting greatly with water. If local regulations prohibit its release then any spillage must be collected for disposal or any spillage absorbed into paper or suitable absorbent and any spent incubation mixture kept in a suitable container for disposal by a licensed waste disposal contractor.

Buffered Glutaraldehyde Reagent –Consult local regulations . In the quantities supplied and used in the test, it is unlikely to pose a significant danger to the environment – therefore dispose of small test quantities to the public sewer diluting greatly with water.Larger spills should be taken up on absorbent granules and disposed of through a licenses waste disposal contractor. Local water authorities may be consulted about local regulations for the release of this substance to the environment (bearing in mind only a small amount is involved < 5% glutaraldehyde). 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.

Haematoxylin counterstain – Mop up with damp cloth. Rinsing cloth with tapwater to the public sewer.

Copper nitrate – dispose of through public sewer diluting greatly with water.

Tris-HCL buffer concentrate – dispose of through public sewer diluting greatly with water.

EA Counterstain – evaporate to atmosphere or dispose of through public sewer diluting greatly with water. Beware of flammable fumes in drains.

Scotts Tapwater substitute – dispose of through public sewer.

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.

GCC Diagnostics Gainland Chemical Co) Factory Road. Sandycroft. Deeside. Flintshire.UK
Tel : 01244 536326 Fax : 01244 531254 email: gball@gccdiagnostics.com www.gccdiagnostics.com
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