

## b-Glucuronidase, lymphocyte

### Use

Cytochemical demonstration lymphocyte beta-glucuronidase activity (focal staining patterns) in buffy coat preparations from blood, bone marrow films, centrifuge preparations or tissue touch preparations are typical of T- lymphocytes. Positive reactions are consistent with lymphocytic leukaemia.

### Principle of Test

After fixation, preparations / films are incubated in a mixture of Naphthol AS-BI-b-glucuronide and hexazotised pararosaniline. b-Glucuronidase activity is visualised by simultaneous capture reactions.. Light microscopy is used to evaluate the staining patters. B-Glucuronidase + hexazotised pararosaniline forms an insoluble red reaction product in the target cells.

### Kit Reagents

1 - Naphthol AS-BI-b-Glucronic acid	50ml
2 - Pararosaniline reagent	25ml
3 - Sodium nitrite reagent	20ml
4 - Citrate solution	50ml
5 - Acetate reagent	50ml
6 - Counterstain reagent	50ml

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 & stability

Store kit components in the kit box provided at cool room temperature in a darkened cupboard. Note expiry date on the pack. 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.  
Deionised water at 37 Deg Celcius for reagent pre and incubation.

### Specimen collection, storage

Blood, bone marrow or tissue touch preparations / films may be used. Samples using Heparin or EDTA anticoagulants are acceptable. Centrifuge and buffy coat preparations are recommended. After fixation slides maybe stored at room temperature for up to 2 weeks without appreciable loss of b-glucuronidase activity. Use appropriate safety procedures in sample collection. All samples should be considered a source of infection.

### Controls

Control slides prepared from cells from healthy donors should also be run at the same time as

the test slides to compare expected resultst to ensure reagent system is working correctly.

### Reagent preparation and Method

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 0.5ml Pararosaniline reagent and 0.5 ml Sodium nitrite reagent. Mix and keep at room temperature for approx 2-3 minutes. Oxides of Nitrogen are produced – work in a fume hood, wear appropriate face mask.
- 3 - In a Coplin Jar or similar add:
  - (a) 5ml Naphthol AS-BI-b-glucuronic acid reagent
  - (b) Content of beaker prepared in step 2.
  - (c) 35ml prewarmed deionised water use a little of the water to rinse beaker and add to Coplin Jar.
  - (d) 5ml Acetate solution
  - (e) Mix well , colour is light brown. Precipitate forming indicated reagent degrading. Maintain temperature at 37DC.
  - (f) Place rinsed slides immediately in incubation mixture and incubate at 37 Deg C for 90 minutes away from direct light.
  - (g) After 90 minutes, remove slides, rinse in tap water for 203 minuted.
  - (h) Allow slides to air-dry for 30-40 minutes.
  - (i) Cover slide with counterstain for 2 minutes.
  - (j) Rinse in deionises water for a few seconds until blue stops running from slide.
  - (k) Examine by light microscopy

### Results

Positive staining is red-brown red water insoluble deposit in focal ( dot) and diffuse-granular patters. Positive stains appears to be associated with circulating T-cells , mature thymocytes and a subpopulation of immature B-cells. Monocytes and granulocytes are usually negative.

### Limitations

All glassware must be thoroughly rinsed with deionised water. Traces of detergent can interfere with such systems. Thoroughly rinse slides after fixation in deionised water. Traces of Formaldehyde can interfere with such 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. Please contact us if your results differ from those expected.

## 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. Some reagents are FLAMMABLE. Keep away from sources of ignition. 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 - Naphthol AS-BI-b-glucuronic acid reagents is FLAMMABLE and TOXIC.

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 and Acetate reagent - No special hazard with these reagents.

5 - Counterstain - Methylene blue reagent may cause irritation to eyes and stains to the skin. Wash with soap & water if stains to the appear. In can also de-oxygenate the blood if ingested in large quantities.

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

## Spillage and 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.

In the case of all the reagents in this kit – mop up spillage with damp cloth, rinse cloth under tapwater diluting to public sewer. Any unused reagent or out of date reagent should be diluted with large quantities of water to the Public Sewer system

## 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.

References: Please contact us for technical reference information.

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