

# BIO-PB<sup>TM</sup> Karyotyping Medium

With phytohemagglutinin-M (PHA-M)

REF 01-201-1



-20°C

### Instructions for Use

### **Product Description**

Biological Industries BIO-PB<sup>TM</sup> Karyotyping Medium is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. BIO-PB<sup>TM</sup> Karyotyping Medium is based on RPMI-1640 basal medium supplemented with L-Glutamine, foetal bovine serum, antibiotics (gentamicin) and Phytohemagglutinin-M (PHA-M).

BIO-PB™ Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

### **Precaution and Disclaimer**

- Do not use if a visible precipitate is observed in the medium.
- Use of Biological Industries BIO-PB™ Karyotyping Medium does not guarantee the successful outcome of any chromosome analysis testing.
- Do not use BIO-PB™ Karyotyping Medium beyond the expiration date indicated on the product label.

### Storage and Stability

- BIO-PB™ Karyotyping Medium should be kept frozen at -20°C.
- After thawing, the medium should be stored at 2-8°C. The medium should be used within 10 days after thawing.
- Protect the medium from light.

### Instructions for Use

- Thaw BIO-PB™ Karyotyping Medium at refrigerator temperatures (2-8°C) or by swirling bottle in a 37°C water bath. Mix gently after thawing.
- Note that the medium already contains L-glutamine, antibiotics, and PHA-M.

## Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

- 1. Inoculate approximately 0.5ml of heparinized whole blood into a glass or plastic tube with 10ml of medium.
- 2. Incubate the culture for 72 hours.
- 3. Add 0.1-0.2ml of Colcemid Solution (Cat. No. 12-004-1) to each culture tube. Incubate the culture for an additional 15-30 minutes.
- 4. Transfer the culture to a centrifuge tube and spin at 500g for 5 minutes.

- 5. Remove the supernatant and re-suspend the cells in 5-10ml of hypotonic **0.075M KCl (Cat. No. 12-005-1)**. Incubate at 37°C for 10-12 minutes.
- 6. Spin at 500g for 5 minutes.
- 7. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5-10ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol. Leave in 4°C for 10 minutes.
- 8. Repeat steps 6 and 7.
- 9. Spin at 500g for 5 minutes.
- 10. Re-suspend the cell pellet in a small volume 0.5-1ml offresh fixative, drop onto a clean slide and allow to air dry.
- 11. At this stage, the preparation can be stained with Orecinor Giemsa. Giemsa banding has become the most widely used technique. The most common method to obtain this staining is to treat slides with Trypsin-EDTA 10X (Cat. No. 03-051-5).

### **Quality Control**

BIO-PB™ Karyotyping Medium is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for karyotyping in a leading clinical cytogenetics laboratory.

### **Quality Assurance**

- For in vitro diagnostic use. The medium is not intended for therapeutic use.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- Manufactured under ISO 13485 QMS.
- Manufactured under controlled environments and processes in accordance with:
  - ISO 13408 Aseptic Processing of Health Care Products
  - 2. ISO 14644 Cleanrooms and associated controlled environments



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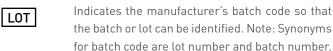


Manufacturer:

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#### **Product Label Symbols**

	REF	Indicates the manufacturer's catalogue number		
so that the product can be identified.		so that the product can be identified.		





Indicates the temperature limits to which the product can be safely exposed.

Indicates a product that has been manufactured using accepted aseptic techniques.

Indicates that the product meets the requirements

of the applicable EC directives

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Indicates a product that is intended to be used as an in vitro diagnostic medical device.

Indicates the need for the user to consult the instructions for use.

### **Related Products**

Product	Cat. No.
Trypsin EDTA, 10X concentrate	03-051-5
Colcemid Solution	12-004-1
0.075M KCl Solution	12-005-1
BIO-PB™ Karyotyping Medium, w/o PHA-M	01-198-1
PHA-M	12-009-1

### References

- Moorhead, P.S., et al, Chromosome Preparations ofLeukocytes Cultured from Human Peripheral Blood, Exp. Cell. Res., 20:613-616 (1960)
- 2. Nowell, P.C., Phytohemagglutinin An Initiator of Mitosisin Cultures of Normal Human Leukocytes, Cancer Res., 290:462-466 (1960)
- 3. Barch, M.J. (ed), The Association of CytogeneticTechnologists Laboratory Manual, Second Edition (1991)

www.bioind.com V4 September 2019 | p.2