BI
Biological Industries

Cytogenetics

Peripheral Blood Karyotyping Medium

With phytohemagglutinin-M (PHA-M)

Cat. No.: 01-201-1
Store at: -20°C

Instructions for Use

Product Description

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

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

Precaution and Disclaimer

For **in vitro** diagnostic use. The medium is not intended for therapeutic use.
Do not use if a visible precipitate is observed in the medium.
Use of Biological Industries PB Karyotyping Medium does not guarantee the successful outcome of any chromosome analysis testing.
Do not use PB Karyotyping Medium beyond the expiration date indicated on the product label.

Storage and Stability

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 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 37ºC 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 4ºC 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 of fresh fixative, drop onto a clean slide and allow to air dry.
11. At this stage, the preparation can be stained with Orecin or 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

PB Karyotyping Medium is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for performance using primary human peripheral blood lymphocytes cultured for 72 hours. The mitotic stimulation is evaluated after chromosomes preparation and staining.

References

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

Related Products

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

BI Biological Industries
Culture of Excellence

Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 25115, Israel
T.+972.4.9960595 F.+972.4.9968896
Email: info@bioind.com
www.bioind.com

CE CERTIFIED QMS ISO 9001 2008 CERTIFIED QMS ISO 13485 2003 cGMP Manufacturing Facility