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Biotechnology

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Karyotyping Medium – PB (with Phytohaemagglutinin)

Catalog No.	IS1097 100 ml
Introduction	ID Labs' Karyotyping Medium - PB is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. This medium is based on RPMI-1640 basal medium supplemented with L-Glutamine, fetal bovine serum, antibiotics (gentamicin) and Phytohemagglutinin (PHA-M).
Format	Karyotyping Medium - PB is supplied frozen and is ready to use after thawing.
Reconstitution	Thaw Karyotyping Medium - PB at refrigerator temperatures (2-8^{o}C) or by swirling bottle in a 37^{o}C water bath. Mix gently after thawing. Note that the medium already contains L-Glutamine, antibiotics, and PHA-M.
Activity	Karyotyping Medium-PB is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for biological performance using primary human peripheral blood lymphocytes. The mitotic index is determined by a colorimetric immunoassay based on the measurement of BrdU incorporation during DNA synthesis.
Use	For short-term cultivation of peripheral blood lymphocytes.
Storage	Store at $\leq$ -18 ^{o}C. Protect from Light. After thawing, this medium should be stored at 2-8 ^{o}C. The medium should be used within 10 days after thawing. Do not use if a visible precipitate is observed in the medium.
Expiry	Do not use beyond expiration date indicated on the product label.

Use of Karyotyping Medium - PB does not guarantee the successful outcome of any chromosome analysis.

Protocol

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 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 at 37^{o}C in 5% CO2 atmosphere for 72 hours.
3. Add 0.1-0.2ml of Colcemid Solution 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 (Catalog# IS1006). Incubate at 37^{o}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^{o}C for 10 minutes.
8. Repeat steps 6 and 7.
9. Spin at 500g for 5 minutes.
10. Resuspend 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, and the most common method to obtain this staining is to treat slides with Trypsin EDTA, 10x (Catalog# IS1008)

Optimal dilution and reaction conditions to be determined by investigator usage.