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Biotechnology

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Karyotyping Medium – PB
(without phytohaemagglutinin)

Catalog No.	IS1003 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, heparin and antibiotics (penicillin and streptomycin).
Format	Karyotyping Medium - PB is supplied frozen and is ready to use after thawing and phytohemagglutinin supplementation.
Reconstitution	Thaw Karyotyping Medium - PB at refrigerator temperatures (2-8^{o}C) or by swirling bottle in a 37^{o}C water bath. Note that the medium already contains L-Glutamine and antibiotics, but not PHA-M.
Activity	Karyotyping Medium-PB is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each lot is tested for cell growth.
Use	For short-term cultivation of peripheral blood lymphocytes.
Storage	Store at -20^{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. Collect peripheral blood sample in a Sodium Heparin tube or syringe.
2. Add 5ml Karyotyping Medium - PB (Catalog# IS1003) supplemented with PHA-M (Catalog# IS1007) to each sterile T-25 flask.
3. Add 0.3-0.4ml blood to each flask.
4. Incubate flasks in CO_2 incubator at 37 ^{o}C for 48-72 hours. Leave the caps loose or closed as desired.
5. Add 0.05ml of Colcemid solution to each flask (final concentration: 0.1 µg/ml). Incubate the culture for an additional 30 minutes.
6. Transfer flask contents to a centrifuge tube and spin at 500-900g for 5 minutes.
7. Remove the supernatant and re-suspend the cells in 5-10ml of hypotonic solution (0.075M KCL, Catalog# IS1006) Incubate at 37 ^{o}C for 10-15 minutes.
8. Centrifuge at 500-800g for 5 minutes.
9. 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.
10. Repeat steps 8 and 9.
11. Spin at 500-800g for 5 minutes.
12. Resuspend the cell pellet in a small volume 0.5-1ml of fresh fixative, drop onto a clean slide and allow to air dry.
13. 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.