

# KREAvital Lymphocyte Karyotyping Medium (with PHA)

**Cat. No:** KBI-90021 (100 ml)

**Store at:** -20°C

## Product Description

KREAvital Lymphocyte Karyotyping Medium is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. It is based on RPMI-1640 basal medium supplemented with L-Glutamine, fetal bovine serum, antibiotics (gentamicin) and Phytohaemagglutinin (PHA-M).

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

## Precaution and Disclaimer

1. For in vitro diagnostic use. The medium is not intended for therapeutic use.
2. Do not use if a visible precipitate is observed in the medium.
3. Use of KREAvital Lymphocyte Karyotyping Medium does not guarantee the successful outcome of any chromosome analysis testing.
4. Do not use KREAvital Lymphocyte Karyotyping Medium beyond the expiration date indicated on the product label.

## Storage and Stability

KREAvital Lymphocyte 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 KREAvital Lymphocyte 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.

## Procedure

1. Inoculate approximately 0.5 ml of heparinized whole blood into a glass or plastic tube with 10 ml of medium.
  2. Incubate the culture at 37°C in 5% CO<sub>2</sub> atmosphere for 72 hours.
  3. Add 0.1 - 0.2 ml of Colcemid Solution (KBI-90051) to each culture tube. Incubate the culture for an additional 15 - 30 minutes.
- Proceed with regular fixation protocol. For specific protocol please enquire.

## Quality Control

KREAvital Lymphocyte 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.*, 29:462-466 (1960)
3. Barch, M.J. (ed), *The Association of Cytogenetic Technologists Laboratory Manual, Second Edition* (1991)

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