LymphoPlus (Peripheral Blood Lymphocyte Karyotyping Medium)
For research use only

Catalogue number: BI-1103

Product description

Peripheral blood lymphocytes are the most common cell source for cytogenetic studies, including detection or confirmation of chromosomal aberrations (numerical and structural changes), autosomal and sex chromosomal anomalies, and their constitutional or somatic effects. This procedure offers the least invasive method for obtaining primary cells for cytogenetic studies.

Lymphocytes are mature, and thus, not actively dividing cells. To induce mitosis in non-dividing cells, a technique was developed by Moorehead et al. in 1960 for the short-term culture of lymphocytes through a mitogen. The most commonly used mitogen is phytohemagglutinin (PHA), which is a red kidney bean extract that transforms small lymphocytes (T cells) into blast-like cells. This transformation is rapidly followed by RNA synthesis with DNA synthesis 24 hours later. Once DNA synthesis begins, the cells are committed and PHA is no longer needed in the culture. Seventy-two hours after the addition of PHA to the culture, about 45% of cells are in the S phase. This represents the peak mitotic activity and is the optimal time to harvest cells for chromosome studies.

Most cytogenetic laboratories cultivate peripheral blood lymphocytes for a period of 48-72 hours in the complete culture medium consisting of a basal medium supplemented with approximately 10-40% Fetal Bovine Serum (FBS), L-glutamine, antibiotics, and PHA in the range of approximately 1-2% v/v, depending on the source. The potency of PHA for mitotic stimulation can vary with different lots. Usually, the optimum concentration usually needs to be determined prior to use.

We have manufactured a completely supplemented and cytogenetically qualified culture medium with consistent performance. LymphoPlus medium (BI-1103) was designed as a complete ready-to-use product and is prequalified on primary cells in an application-specific cytogenetic assay, which is performed by expertized cytogeneticists using standardized protocols. LymphoPlus Medium is prepared based on the universal basal medium, supplemented with FBS, antibiotics (gentamicin), and phytohaemagglutinin (PHA-M). The medium is intended for use in in vitro procedures, which requires the short-term cultivation of peripheral blood lymphocytes for cytogenetic studies.

Instructions for use

1. Warm the medium to room temperature and gently swirl to mix prior to use.
2. Inoculate approximately 0.5 ml of heparinized whole blood into a glass or plastic tube with 5-10 ml of the medium.
3. Incubate the culture at 37ºC in 5% CO2 atmosphere for 72 hours.
4. Add 0.1-0.2 ml of colchicine solution to each culture tube. Incubate the culture for an additional 15-30 minutes.
5. Proceed with regular fixation protocol.

Storage and handling

- LymphoPlus should be kept frozen at -20 °C and after thawing, the medium should be stored at 2-8 °C.
- Note that the medium already contains antibiotics, FBS and PHA-M. Do not thaw at 37°C.
- The medium should be used within 10 days after thawing and storing at 2-8 °C.
- LymphoPlus can be thawed and aseptically transferred into smaller aliquots for convenience. These aliquots can be frozen and thawed at the time of use. However, multiple freeze-thaw cycles should be avoided.
- Avoid prolonged exposure to light.
- LymphoPlus is provided as a 1X liquid complete medium that is ready to use once thawed. This design allows for optimal shelf life and requires no additional supplementation, which minimizes end-user handling.

Do not use, if

- Medium appears cloudy.
- Package has been compromised.
- Product was received completely thawed.
- Visible precipitate is observed in the medium.
- The expiration date is passed.

Quality Control and limitations

1. LymphoPlus medium is tested for sterility, pH, and endotoxin concentrations. In addition, batches are routinely tested for performance using primary human peripheral blood lymphocytes cultured for 72 hours.
2. Although this media will likely perform for use in propagation of other cell types, their intended use is not subject to present quality control procedures and no assurances are presently given for their application.
3. Each clinician/scientist/researcher must make an independent judgment on whether this medium is suitable for use in their laboratory.

Important notes:

- The medium is not intended for diagnostic or therapeutic use.
- For research use only.

Related Products:

In order to synch cell cycle before karyotyping in a heterogeneous population, use other products including Lymphocyte Karyotyping Kit (BI-2001), Lymphocyte HR Karyotyping Kit (BI-2002), and Cell Synchronization Kit (BI-2003).

References:

1. Kelly, T.E., 1986. Clinical genetics and genetic counselling.
2. Knutsen, T., 1992. International Cytogenetic Laboratory Directory. Association of Cytogenetie Technologists, Burbank, California.
3. Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.T. and Hungerford, D.A., 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Experimental cell research, 20(3), pp.613-616.
4. Barch, M.J., The association of cytogenetic technologists laboratory manual. New York: Act.
5. Nowell, P.C., 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer research, 20(4), pp.462-466.