UAB "LT Biotech"

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LymphoFirst
Complete Medium for Peripheral Blood Lymphocytes
Cat. No.: AF100ML (100 ml)

General Information

LymphoFirst Medium is intended for use in short-term cultivation of peripheral blood lymphocytes for chro-mosome evaluation. The medium is based on a basal medium supplemented with L-glutamine, fetal bovine serum, antibiotics (gentamicin) and phytohemagglutinin-M (PHA-M). It is supplied as frozen medium, which is ready for use after thaw-ing.

Product Specifications

Appearance	Clear yellow to red frozen liquid
Storage and shelf life	Store at ≤-15°C protected from light. Do not use this product after its expiry date. Once opened, store at +2°C to +8°C and use within 10 days.
Shipping conditions	Frozen (Dry Ice)

For lot specific data (**Certificate of Analysis**) please refer to our website: www.capricorn-scientific.com/products/

Thawing

Thaw LymphoFirst Medium at refrigerator temperatures (+2°C to +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.

Instructions for Use

Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

Blood cell karyotyping of lymphocytes is an important tool in modern human cytogenetics to detect chromosomal abnormalities. Lymphocytes usually do not undergo subsequent cell divisions. In the presence of a mitogen (e.g. PHA), lymphocytes are stimulated to enter into mitosis. After 48 – 72 hours, a mitotic inhibitor (e.g. colcemid) is add-ed 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. Thaw LymphoFirst Medium and make aliquots of 10 ml (sterile tubes).
2. Thaw the pre-calculated amount of LymphoFirst Medium (in tubes) until room temperature is reached.
3. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml LymphoFirst Medium.
4. Incubate the culture at +37°C, 5% CO_2 in an incubator for 72 hours.
5. Add 0.1 – 0.2 ml of Colcemid Solution (Cat. No. COL-H) to each culture tube (at a final concentration of 0.1µg/ml). Incubate the culture for additional 15 – 30 minutes.
6. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.

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7. Remove the supernatant and re-suspend the cells in 5 – 10ml of hypotonic 0.075 M KCl, pre-warmed to +37°C. Incubate at +37ºC for 10 – 12 minutes.
8. Spin at 500 g for 5 minutes.
9. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5 – 10 ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol. Leave at + 4ºC for 10 minutes.
10. Repeat steps 8 and 9.
11. Spin at 500 g for 5 minutes.
12. Re-suspend the cell pellet in a small volume 0.5 – 1 ml 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. The most common method to obtain this staining is to treat slides with Trypsin-EDTA 10x (Cat. No. TRY-1B10).

Precautions 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 LymphoFirst Medium does not guarantee the successful outcome of any prenatal diagnostic testing.
Do not use LymphoFirst Medium beyond the expiration date indicated on the product label.