

## UAB "LT Biotech"

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### **LymphoFirst2** **Complete Medium for Peripheral Blood Lymphocytes** **Cat. No.: AF200ML (100 ml)**

#### **General Information**

LymphoFirst2 is a recently developed and enhanced medium that has been formulated with extensive expertise to provide superior performance and longer stability. It has been specifically designed for the temporary growth of peripheral blood lymphocytes for chromosome analysis. The composition of LymphoFirst2 includes L-glutamine, pre-screened fetal bovine serum (FBS), antibiotics, phytohemagglutinin-M (PHA-M), and a carefully calibrated concentration of growth factors to promote cell growth.

#### **Product Specifications**

Appearance	Clear yellow to red frozen liquid
Storage and shelf life	Store at $\leq -15^{\circ}\text{C}$ protected from light. Do not use this product after its expiry date. Once opened, store at $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$ 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/](http://www.capricorn-scientific.com/products/)

#### **Thawing**

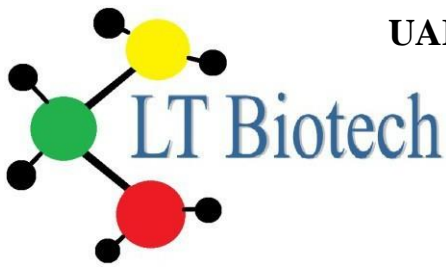
Thaw LymphoFirst Medium at refrigerator temperatures ( $+2^{\circ}\text{C}$  to  $+8^{\circ}\text{C}$ ) or by swirling bottle in a  $+37^{\circ}\text{C}$  water bath. Mix gently after thawing.  
Note that the medium already contains L-glutamine, antibiotics, and PHA-M.

#### **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 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. Thaw LymphoFirst2 Medium and make aliquots of 10 ml (sterile tubes).
2. Thaw the pre-calculated amount of LymphoFirst2 Medium (in tubes) until room temperature is reached.
3. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml LymphoFirst2 Medium.
4. Incubate the culture at  $+37^{\circ}\text{C}$ , 5%  $\text{CO}_2$  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 0.1  $\mu\text{g}/\text{ml}$ ). 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 +37°C 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 + 4°C for 10 minutes.
10. Repeat steps 8 and 9.
11. Spin at 500 g for 5 minutes.
12. Resuspend 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 Orcein or Giemsa. For Giemsa staining, the most widely used method, you can use one of the common staining protocols or the protocol established in your laboratory.

### 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 LymphoFirst2 Medium does not guarantee the successful outcome of any prenatal diagnostic testing. Do not use LymphoFirst2 Medium beyond the expiration date indicated on the product label.