Product Information, Instructions for Use

LymphoPrime, Complete Medium for Peripheral Blood Lymphocytes
Cat. No.: LPR-B (100 ml)

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Product Description

LymphoPrime Medium is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome 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 thawing.

Product Specifications

pH	7.0 – 7.5
Endotoxin	<25 EU/ml
Lymphocyte Cell Growth	Positive
Mycoplasma	Not detected
Sterility	Tested
Storage	Store at ≤-15°C. After thawing, the medium should be stored at +2°C to +8°C. The medium should be used within 10 days after thawing. Protect the medium from light.

Thawing

Thaw LymphoPrimet 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 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 LymphoPrime Medium and make aliquots of 10 ml (sterile tubes).
2. Thaw the pre-calculated amount of LymphoPrime Medium (in tubes) until room temperature is reached.
3. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml LymphoPrime 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.
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. 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).

Related Products

Product	Cat. No.
Colcemid Solution (10 µg/ml) in DPBS	COL-H
Trypsin-EDTA (0.5 %) in DPBS (10x)	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 LymphoPrime Medium does not guarantee the successful outcome of any chromosome analysis testing.
- Do not use LymphoPrime Medium beyond the expiration date indicated on the product label.

Help needed?

If you have any further questions regarding this product please do not hesitate to contact our cell culture experts:
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