LYMPHOGROW

Complete Karyotyping Medium for Peripheral Blood Lymphocytes, Sterile-filtered

Filtration, Treatment	Sterile Filtered; Contains preselected FBS, L-Glutamine and antibiotics.
Product Code	LMG-001-100ML
Shelf Life	24 months from DOM
Storage Temperature	Store between-5°C to -20°C protected from light. Once opened, store at +2°C to +8°C and use within 2 weeks
Shipping Temperature	Frozen (Dry ice)
Thawing	Thaw the medium at 2°C to 8°C, or alternatively at 37°C in water bath and swirl gently to homogenize
CO2 concentration, optimum	5 %

QC Specifications

Physical and Chemical Analysis	**Method**	**Specifications**	**Units**
Appearance	Visual	Clear amber to red frozen liquid	n/a
pH at RT	Electronic pH Meter	6.8 - 7.6	n/a
Osmolality	Osmometer	Test and report	mOsm/kg
Endotoxin	LAL Kinetic	≤ 10.0	EU/ml
Sterility			
Aerobic Bacteria	EP 2.6.1	Not detected	n/a
Anaerobic Bacteria	EP 2.6.1	Not detected	n/a
Fungi (Yeast & Mold)	EP 2.6.1	Not detected	n/a
Mycoplasma	qPCR	Not detected	n/a
Functionality Test	Internally Validated	Pass	n/a

GENERAL INFORMATION/FORMULATION

LymphoGrow™ formulation has been developed and optimized by Serana R & D team and its superiority over the commercially available similar products were approved for the cytogenetics-related parameters in short-term and long-term cultivation of Peripheral Blood Lymphocytes, which are intended for the preparation of karyograms, fluorescence *in situ* hybridization and other cytogenetic methods. The medium is supplied frozen.

Important information:

This medium is ready to use and no further supplements are needed. *LymphoGrow™* is formulated based on the basal medium and already supplemented with preselected Foetal Bovine Serum, L-Glutamine and antibiotics.

INSTRUCTION FOR USE

Lymphocytes are a subtype of leukocytes substantial for immunoregulation and play an important role in the development of promising and feasible immunotherapeutic cancer therapies. Karyotyping of peripheral blood lymphocytes (PBL's) has been proved to be an inalienable tool for characterization of the complex chromosome rearrangements in leukemia (68). The determination of leukemia specific cytogenetic abnormalities provides insight into leukemia pathogenesis and allows integral prognostic assessment.

Lymphocytes usually do not undergo subsequent cell divisions. In the presence of a mitogen, inducer of the cell division, lymphocytes are triggered to enter into mitosis. After 48 – 72 hours, colcemid is added to the culture as the mitotic inhibitor, arresting mitosis on the metaphase stage by interfering with the formation of spindle apparatus. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

1. We recommend to initially thawing *LymphoGrow™* Medium per described and making aliquots of 10 ml sterile tubes.

2. Thaw the precalculated amount of *LymphoGrow*^{TM} per described.
3. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml *Lymphorow* Medium.
4. Incubate the culture at +37°C, 5 % CO_2 in an incubator for 72 hours.
5. Add Colcemid Solution (Product Code: *CDS-002-10ML*) to each culture tube at a final concentration of 0.1 µg/ml; 0.1 ml in each 10ml of medium).
6. Incubate the culture for additional 30-60 minutes.
7. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
8. Remove the supernatant and resuspend the cells in 5 ml of hypotonic 0.075 M KCl (Product Code: *CDS-003-100ML*), prewarmed to +37°C. The hypotonic solution allows swelling of the cells to increase the visibility of chromosomes.
9. Incubate at +37ºC for 10 minutes.
10. Spin at 500 g for 5 minutes.
11. Resuspend the cell pellet in a 5 ml of an ice-cold fixative (1:3 solution of AcOH and MeOH) dropwise. The fixative kills the cells, removes cell debris and helps to preserve cellular structures.
12. Leave at + 4ºC for 10 minutes.
13. Repeat steps 10-12.
14. Spin at 500 g for 5 minutes and discard the supernatant.
15. Resuspend the cell pellet in a small volume (0.5 – 1 ml) of fresh fixative, drop it onto a clean slide, and allow it to air dry.
16. stain the cells with Orecin or Giemsa using protocol established in your laboratory.

PRECAUTIONS AND DISCLAIMER

The medium is not intended for therapeutic use.
Each laboratory is obliged to perform representative tests according to valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the medium can be used in routine diagnostics.
Do not use if a visible precipitate is observed in the medium.
Do not use this medium beyond the expiration date indicated on the product label.