



LymGro® Serum-free Lymphocyte Culture Medium containing Human serum albumin (HSA) and Phenol red-free

Cat.No	Product	Amount	Shelf Life	Type	Storage	Transportation
T120KJ	LymGro® Serum-free Lymphocyte Culture medium containing HSA, Phenol red-free	500 mL	12 Months	Liquid	2 ~ 8 °C, Avoid Light	Blue Ice

1.Introductions

LymGro® Lymphocyte Medium is a serum-free medium specifically designed for tumor immunotherapy research. The product is suitable for the culture of human and mammalian peripheral blood immune cells, including the research and application of T cells, DCs, CIK cells, NK cells, and others. By adding different cell-stimulating cytokines, the corresponding immune cells can be efficiently and rapidly expanded. It can also be used to culture functionally differentiated lymphocyte lines, study signal transduction pathways, and perform applications with defined component requirements. Application areas of this medium include: chimeric antigen receptor T lymphocyte-related research (CAR-T); induction and functional studies of lymphokine-activated killer (LAK) cells; cytokine and antibody activity studies during LAK cell induction; long-term culture of functional human macrophages and macrophage activation; murine liver metastasis immunotherapy research; lymphocyte receptor research; and ADCC-dependent lymphocyte activation research, etc.

This product is prepared using Water for Injection (WFI).

Product Highlights

Contains (+):

- Human serum albumin (HSA)
- D-glucose
- L-glutamine

Does not contain (-):

- Phenol red

2. Quality management system

The product is manufactured in CGMP standard workshop in Basalmedia Shanghai.

Basalmedia Shanghai Biotechnology Co.Ltd has obtained ISO9001 :2015 , ISO13485 :2016 quality system certification.

3. Product parameters

The product is sterilized by filtration.

Appearance: Transparent liquid with red color

Endotoxin: ≤3 EU/mL

Osmotic pressure: 280 ~ 330 mOsm/kg·H₂O

pH value: 7.0 ~ 7.4

Storage: 2 ~ 8 °C, Avoid Light

Transportation: Blue Ice

Application: for research and production only

4.User Guide

Preparation of Medium

LymGro® Lymphocyte Serum-Free Medium contains L- glutamine. For different applications or sample requirements, appropriate cytokines should be added to the medium before use (the medium should be used immediately after cytokines are added).

Normal saline used during the experiments can be replaced with Dulbecco' s Phosphate-Buffered Saline (DPBS) without calcium and magnesium.

Cell Culture Conditions

Medium: LymGro® Lymphocyte Serum-Free Medium

Cell lines: human and mammalian peripheral blood immune cells.

Cell type: suspension cells (static suspension culture)

Culture vessels and equipment: culture flasks, culture bags, and a CO₂ incubator

Culture conditions: 36–38 °C, humidified air with 4–6% CO₂, protected from light.

Isolation of PBMCs

1. Collect the peripheral blood into two sterile centrifuge tubes. Centrifuge at 2000 rpm for 5 min, discard the supernatant, and add normal saline to 25 mL to fully resuspend the cell pellet. Take another centrifuge tube and add 20 mL of lymphocyte separation medium. Using a pipette, slowly transfer the blood cell suspension onto the surface of the lymphocyte separation medium, forming a clear interface between the two layers.

2. After centrifugation at 2000 rpm for 20 min, four layers will form from the bottom to the top: the red blood cell and granulocyte layer, the lymphocyte separation medium layer, the peripheral blood mononuclear cell layer (PBMC layer), and

the plasma layer. Aspirate the PBMC layer with a pipette and transfer it to another sterile centrifuge tube.

3. Add DPBS to 40 mL, centrifuge at 1500 rpm for 5 min, discard the supernatant, and repeat this step 3 times.

4. After the final centrifugation, discard the supernatant, add 40 mL of LymGro® Lymphocyte Serum-Free Medium, and culture under the recommended culture conditions.

Cell culturing procedure

The following procedure serves as a general method for static culture of immune cells.

Maintain and feed the cells during the logarithmic growth phase to reach the desired cell number. Whenever the viable cell density reaches 2×10^6 cells/mL, dilute the culture volume 1:1 with fresh medium and continue culturing.

To ensure adequate gas exchange, it is recommended that the medium depth does not exceed 1.2 cm (optimal depth 1–1.2 cm).

For high-density culture in bioreactors or culture bags, conditions should be optimized to determine the optimal operating steps.

T cell culturing

1. Prepare PBMCs according to the PBMC isolation method and perform a cell count.

2. At the initial stage of culture, resuspend PBMCs in pre-warmed LymGro® Lymphocyte Serum-Free Medium containing cytokines (such as IL-2) at a viable cell density of approximately $0.5\text{--}1 \times 10^6$ cells/mL. Transfer the cells to an appropriate cell culture vessel and place in the incubator.

Note: Subsequently, various methods can be applied to activate T cells, including the addition of stimulatory antibodies or antigen-presenting cells. Regardless of the scale of T cell culture, cells can be isolated, activated, and expanded.

Monocyte-Derived Dendritic Cell (DC) Culturing

1. Prepare PBMCs according to the PBMC isolation method.

2. After cell isolation, place the cells in the incubator and culture for 2–3 hours.

3. Discard the medium containing non-adherent cells.

4. Wash the adherent cells (mainly CD14⁺ monocytes) three times with normal saline.

5. Add recombinant human IL-4 and recombinant human GM-CSF to LymGro® Lymphocyte Serum-Free Medium to final

concentrations of 50–100 ng/mL and 50 ng/mL, respectively, and culture the adherent cells with this medium. Maintain the cell density between $1\text{--}3 \times 10^5$ cells/cm².

6. Culture continuously in the incubator for 5 days. It is recommended to replace the medium around day 3 with pre-warmed LymGro® Lymphocyte Serum-Free Medium supplemented with IL-4 and GM-CSF. During the medium change, all cells must be retained. Transfer the liquid from the culture vessel to a 15 mL sterile centrifuge tube (adherent cells will still remain in the vessel). Centrifuge at $200 \times g$ for 5–10 min, aspirate the supernatant, then resuspend the cell pellet in a volume of pre-warmed fresh medium equal to the supernatant volume, and transfer the cell suspension back into the original culture vessel containing the adherent cells. Continue culturing.

7. After 6 days, the cells will exhibit typical dendritic cell morphology and surface markers (e.g., CD1a, CD80, CD86, HLA-DR).

8. Add 1 ng/mL LPS or 50 ng/mL TNF- α to LymGro® Lymphocyte Serum-Free Medium to induce dendritic cell maturation.

9. When the cells reach the required number, centrifuge and collect the cells for use.

Note: During DC culture, if the medium turns yellow, change the medium and supplement with the relevant cytokines.

Cytokine-Induced Killer (CIK) Cell Culturing

1. Prepare PBMCs according to the PBMC isolation method. After the final centrifugation and removal of the supernatant, resuspend the cells in a small volume of pre-warmed LymGro® Lymphocyte Serum-Free Medium containing IFN- γ (1000 U/mL) and perform a cell count.

2. Seed the cells at a viable cell density of $1\text{--}2 \times 10^6$ cells/mL in the above medium.

3. Place in the incubator and culture for 24 hours.

4. Add IL-1 α (0.1 U/mL), anti-CD3 monoclonal antibody (50 ng/mL), and recombinant human IL-2 (0.3 U/mL), and continue culturing. Every 3 days, add fresh pre-warmed LymGro® Lymphocyte Serum-Free Medium and then supplement with IL-2 (0.3 U/mL), maintaining the viable cell density at $0.5\text{--}1 \times 10^6$ cells/mL.

5. On days 5, 7, and 9, take cell samples for phenotypic analysis (FCM) and cytotoxicity assay (MTT assay).

6. When the cells reach the required number, centrifuge and collect the cells for use.

Note: During CIK culture, if the culture medium turns yellow, change the medium and supplement with IL-2 after the medium change. If undesirable flocculent cell clumps are observed, collect the cells in a centrifuge tube, let stand for approximately 5 min to allow the clumps to settle, and then carefully transfer the supernatant cell suspension back into the culture flask.

DC-CIK Co-culture

1. Prepare PBMCs according to the PBMC isolation method. After the final addition of LymGro® Lymphocyte Serum-Free Medium, transfer the cells into three sterile culture vessels, labeled A1, A2, and A3.
2. Place in the incubator and culture for 2–3 hours.
3. Take out the cultured cells and transfer the suspended lymphocytes from A1, A2, and A3 into three separate sterile culture vessels, labeled B1, B2, and B3, respectively. Process the A flasks according to steps 4–8 of the DC culture method described above; process the B flasks according to steps 1–4 of the CIK culture method described above.
4. On day 8, detach the DCs from the A flasks, collect them, and mix with the corresponding CIK cells for co-culture. Discard the A flasks. If the total cell number is very large, the mixture of DCs and CIK cells can be equally divided into the A and B flasks.
5. On day 10, perform microbiological testing on the co-cultured DC-CIK cells.
6. When the cells reach the required number, centrifuge and collect the cells for use.

Natural Killer (NK) cell culturing

1. Prepare PBMCs according to the PBMC isolation method. After the final centrifugation and removal of the supernatant, resuspend the cells in a small volume of pre-warmed LymGro® Lymphocyte Serum-Free Medium containing NK cell activation and proliferation factors, perform a cell count, and then seed the cells at a viable cell density of $1-2 \times 10^6$ cells/mL in the above medium.
- Note: Relevant factors include anti-CD3, IL-2, IL-4, IL-5, IL-12, IL-15, and retronectin. Among these, anti-CD3, IL-2, IL-4, and IL-12 are mandatory factors; concentrations should be adjusted according to the specific situation.
2. On day 3 of culture, centrifuge (1500 rpm, 5 min) and replace with fresh medium and relevant cytokines.
 3. On days 4–6, observe and count the cells under a microscope daily. Add culture medium according to the color

of the cell suspension; each addition should be approximately one-third of the total volume.

4. On day 7, count the cells. When the cell number exceeds 6×10^7 , add expansion reagent. If the cell number is $3-6 \times 10^7$, continue culturing until day 8 and then add NK expansion reagent. When adding medium, maintain the total cell concentration at $1-1.5 \times 10^6$ cells/mL.
5. When the culture volume exceeds 150 mL or the cell number exceeds 1.5×10^8 , transfer the culture to a culture bag.
6. On days 8–11, observe and count the cells under a microscope daily, and add culture medium according to the color of the cell suspension. On days 12–14, when medium supplementation is needed, maintain the cell concentration at 2×10^6 cells/mL.
7. When the cells reach the required number, centrifuge and collect the cells for use.

Chimeric Antigen Receptor T (CAR-T) Cell Culture

Because CAR-T cells are genetically modified T cells, cell culture is part of the overall CAR-T cell experiment. This section mainly describes the general experimental procedure for culturing T cells that stably express CAR. For other CAR-T-related procedures, only general descriptions are provided here:

1. Select the target: identify known tumor-specific antigens (or tumor-associated antigens) or discover new antigens.
2. Obtain scFv: immunize mice, construct a gene library, and screen for high-affinity scFv.
3. Construct CAR-T cells: construct a CAR expression vector, isolate T cells (refer to the experimental methods described above), transfect, and select T cells that stably express CAR.
4. Culture of stably expressing CAR-T cells:
 - ① Perform a cell count.
 - ② Resuspend CAR-T cells in pre-warmed LymGro® Lymphocyte Serum-Free Medium containing cytokines (such as IL-2) at a viable cell density of approximately $0.5-1 \times 10^6$ cells/mL. Transfer the cells to an appropriate cell culture vessel and place in the incubator.
 - ③ Every 3 days, add fresh pre-warmed LymGro® Lymphocyte Serum-Free Medium and then supplement with IL-2 (0.3 U/mL), maintaining the viable cell density at $0.5-1 \times 10^6$ cells/mL.

5. In vitro validation of CAR-T cells: When the cells reach the required number, perform cytokine expression profiling and target tumor cell lysis assays.

6. After validation meets the requirements, collect the cells for subsequent use.

Cell Suspension Preparation

Centrifuge (1500 rpm, 5 min) to collect the cells and discard the supernatant. Wash the cells with normal saline containing 0.5–1% albumin and repeat 3 times. Finally, resuspend the cells in a small volume of resuspension solution, perform a cell count, and then adjust to the appropriate volume with the required cell density to prepare the cell suspension.

5.Related Products

Cat.No.	Product	Amount	Storage	Transportation
T110KJ	LymGro® Serum-free Lymphocyte Culture Medium containing HSA	500 mL	2 ~ 8 °C	Blue Ice
T130KJ	LymGro® Serum-free Lymphocyte Culture Medium, Animal Component and Phenol red-free	500 mL	2 ~ 8 °C	Blue Ice
R710JV	Sample Density Separation Medium, 1.077	100 mL	2 ~ 30 °C	RT
S210JV	L-Glutamine Solution, 100X *	100 mL	-30 ~ -5 °C	Dry Ice
S240JV	L-Alanyl-L-Glutamine Solution, 100X *	100 mL	2 ~ 8 °C	Blue Ice
B210KJ	Dulbecco's DPBS, without Ca ²⁺ , Mg ²⁺ , Phenol red	100 mL	2 ~ 30 °C	RT
B310KJ	Phosphate-Buffered Saline (PBS), pH7.2	500 mL	2 ~ 30 °C	RT
B320KJ	Phosphate-Buffered Saline (PBS), pH7.4	500 mL	2 ~ 30 °C	RT

* 100X indicates that the product concentration is 100 times the working concentration.