

## Introduction

The Serum-free medium for lymphocyte is an upgraded medium developed based on the L100 serum-free medium for lymphocyte and applicable to in vitro culture of human lymphocytes. This product is a chemically defined medium, all the compositions of which are produced from cell culture grade materials. This product contains no heterogeneous animal component or serum or any other protein component except albumin, transferrin and insulin and can effectively avoid the influence of serum qualitative variation, serum components and exogenous components on experimental research and achieve the high-density culture of immunocytes such as human lymphocytes, cytokine induced killer cells, natural killer cells and dendritic cells.

## Package Information

Component	C0126
Serum-Free Medium for Lymphocyte	1000 ml

## Storage

Store at 4 °C and protect from light.

## Applications

The Serum-Free Medium for Lymphocyte supports the high-density suspension culture of lymphocytes. It supports the suspension culture of lymphocytes with the density of  $5 \times 10^5 \sim 5 \times 10^6$  cells/mL in a static culture container. To ensure sufficient gas exchange of the culture system, the recommended depth of the medium is 1~1.5 cm. The medium also supports the high-density culture of lymphocytes in a bioreactor, and the optimization of the culture procedure should be determined by researchers based on experience. The product supports the serum-free culture of immunocytes, and the addition of autologous plasma, human AB serum, FBS or serum substitutes can significantly improve the cell viability and increase the cell proliferation rate. The cell culture supplemental mix is recommended to obtain the optimal culture result.

## Protocol

1. Place the serum-free medium for lymphocyte at room temperature for equilibrium. The following operations shall be in an aseptic condition:
2. Separate lymphocytes of human peripheral blood in an aseptic condition.
3. Inoculate PBMCs obtained by separation to a T75 or T175 cell culture bottle with the serum-free medium for lymphocyte at a concentration of  $2 \sim 2.5 \times 10^6$  cells/ml, add recombinant human IFN- $\gamma$ , etc. , and culture in a 5% CO<sub>2</sub> incubator at 37°C.

## Serum-Free Medium for Lymphocyte

Cat. #: C0126 Size: 1000 ml

4. After 24 hr, i.e., on the first day, add CD3 monoclonal antibody, recombinant human IL-2, etc. to stimulate growth and proliferation of cytokine induced killer cells.
5. From the fourth day, sample every two days to calculate the cell concentration, supplement fresh serum-free medium for lymphocyte based on the calculation result, and adjust the cell concentration to  $1.5 \times 10^6$  cells/ml.
6. On the seventh day, change a bottle with a larger volume or transfer into a cell culture bag according to the volume of the cell culture suspension. The maximum culture volume of a T75 culture bottle is 40 ml, and the maximum culture volume of a T175 culture bottle is 200 ml. When the volume of the medium is more than 200 ml, transfer the cell culture suspension into a cell culture bag.
7. Cell proliferation and cell surface marker detection.