



# **HIPP-X100 Lymphocyte Serum-free Medium**

**Product Name: HIPP-X100**

## **User Manual**

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## Description

HIPP-X100 Lymphocyte Serum-free Medium is a maintenance and expansion medium designed, developed and produced by Shanghai BioEngine Sci-Tech Co., Ltd. specifically for T cell and NK cell culture. It is serum-free, xeno-free, cytokine-free, and phenol red-free. This medium is suitable for the cultivation of various types of T cells and NK cells, as well as perfusion culture processes, without the need to add serum or serum substitutes. It needs to be used in conjunction with HIPP-X100 Lymphocyte Serum-free Medium Supplement (see "Related Products" for details) to obtain high-performance of T cell and NK cell expansion ability and maintain high viability.

## Application

HIPP-X100 is suitable for general culture of T cells,  $\gamma\delta$ T, TIL, CAR-T, TCR-T, CTL, NK, NK-92, NK-92MI, Hematopoietic stem cells, Jurkat cells and K562 and other cells.

This product is intended for research or further manufacturing in the bio-manufacturing industry, but not for human or therapeutic use.

## Storage

- Store HIPP-X100 at 2-8°C, away from light.

- Store HIPP-X100 supplement at -20°C

*Note:*

<sup>[1]</sup> HIPP-X100 complete medium is a serum-free medium designed specifically for the cultivation of T cells and NK cells. It does not require the addition of serum or serum substitutes. If the user chooses to add it, the amount must be determined based on the specific type of T cells or NK cells being cultured.

## Preparation of Complete Medium

- 1) Open the lid of the HIPP-X100 lymphocyte serum-free medium and HIPP-X100 lymphocyte serum-free medium supplement in the biosafety cabinet. Add 5 mL of supplement per 1 L of HIPP-X100 (or 1.25 mL per 250 mL). Tighten the lid of the HIPP-X100 lymphocyte serum-free medium bottle, invert it 3 to 5 times to mix thoroughly. The resulting mixture is HIPP-X100 complete medium. Balance it to room temperature before use. Store it in a dark environment at 2-8°C after use.

***Note:*** It is recommended to prepare HIPP-X100 Complete Medium fresh for immediate use. Prepared complete medium should be used within one week. After each use, store it at 2-8°C, protected from light.

- 2) Additional cytokines such as IL-2, IL-7, and IL-15 must be added to HIPP-X100 complete medium to support the growth and expansion of T cells or NK cells. The types and concentrations of cytokines added should be adjusted appropriately according to actual experimental conditions for optimal results. It is recommended to use HIPP-X100 complete medium with added cytokines within one week.

## Cell Culture (T cells) in Well Plates, Flasks, and Plates

- 1) Prepare fresh PBMCs or frozen PBMCs and quickly thaw them in a 37 °C water bath according to standard thawing procedures.

*Note : If T cells are needed as the seeding cells, they can be sorted from PBMCs, and there are various commercial T cell sorting reagents available for selection.*

- 2) Wash the cells by resuspension in HIPP-X100 complete medium that has been equilibrated to room temperature, centrifuge at  $200-300 \times g$  for 5-10 minutes, and discard the supernatant.
- 3) Use HIPP-X100 complete medium to resuspend cells, with viable cell density controlled between  $0.5-1 \times 10^6$  cells/mL.
- 4) Transfer cells to culture containers with various options such as well plates, plates, and flasks. Add corresponding T cell activation factors, such as CD3 antibodies and CD28 antibodies or anti-CD3/CD28 magnetic beads, according to the recommended usage method by the supplier.

*Note : If free anti-CD3 and anti-CD28 antibodies for activation are used, it is recommended to coat the culture container with both of them at  $2-8 \text{ }^\circ\text{C}$  overnight one day in advance before use. The recommended coating concentration is  $0.5-5 \mu\text{g/L}$ , which can be optimized as needed to obtain the optimal concentration. If CD3/CD28 coupled magnetic beads are used, they should be added on the day of seeding according to the recommended ratio by the supplier.*

- 5) The inoculated cells were cultured in a  $37 \text{ }^\circ\text{C}$ , 5%  $\text{CO}_2$ , saturated humidity incubator.

*Note : On the first or second day after seeding, lentiviruses or retroviruses carrying the target gene can be added for transduction.*

- 6) Starting from the third day, every 1-2 days, determine the viable cell density and adjust it to the range of  $0.5-1.0 \times 10^6$  cells/mL by adding fresh HIPP-X100 complete medium with added cytokines. When the culture volume is expanded to a sufficient size, cells can be transferred to a larger culture

container for further cultivation, such as a larger flask, cell culture bags, etc.

*Note : The cultivation of NK cells can be classified into cytokine based method and feeder cell based method. HIPP-X100 complete medium is suitable for both methods, and appropriate NK cell cultivation methods can be used according to the experimental purpose.*

### **G-Rex Perfusion(T cells)**

- 1) Prepare fresh PBMCs or frozen PBMCs and quickly thaw them in a  $37 \text{ }^\circ\text{C}$  water bath according to standard thawing procedures.

*Note : If T cells are needed as the seeding cells, they can be sorted from PBMCs, and there are various commercial T cell sorting reagents available for selection.*

- 2) Wash the cells by resuspension in HIPP-X100 complete medium that has been equilibrated to room temperature, centrifuge at  $200-300 \times g$  for 5-10 minutes, and discard the supernatant.
- 3) Use HIPP-X100 complete medium to resuspend cells, with viable cell density controlled between  $0.5-1 \times 10^6$  cells/mL.
- 4) Transfer the cells to G-Rex and inoculate them at a cell density of  $0.5-1.0 \times 10^6$  cells/cm<sup>2</sup>. Add appropriate T cell activation factors, such as CD3 antibodies and CD28 antibodies or anti-CD3/CD28 magnetic beads, according to the recommended usage method by the supplier.
- 5) After inoculation, culture the G-Rex in a  $37 \text{ }^\circ\text{C}$ , 5%  $\text{CO}_2$ , and saturated humidity incubator.
- 6) Starting from the 2nd or 3rd day, leveraging the excellent gas permeability of the bottom of G-Rex, add fresh HIPP-X100 complete medium with cytokines directly to the maximum culture volume of G-Rex.
- 7) Afterwards, replenish cytokines (such as IL-2) every two days. Due to the sufficient nutrient supply

provided by the culture medium in G-Rex for cell growth, it is recommended to replace 3/4 of the maximum culture volume with fresh medium every 4 days.

- 8) Sample and count every 1-2 days, the maximum supported cell density for G-Rex is approximately  $2-4 \times 10^7$  cells/cm<sup>2</sup>. When the cell density reaches this range, it is recommended to transfer the cells to a larger scale of G-Rex or harvest the cells for subsequent applications.

*Note: The cultivation of NK cells can be classified into cytokine based method and feeder cell based method. HIPP-X100 complete medium is suitable for both methods, and appropriate NK cell cultivation methods can be used according to the experimental purpose.*

Recovery of the cell membrane In serum-free HIPP-X100 medium may be slower than in serum containing medium. Therefore, it is recommended to extend the time between activation and transduction by 1-2 days compared to serum containing culture systems. This allows sufficient time for the cell membrane to be repaired to a better state before transduction.

## Recommendations for Cell Culture

- 1) Recommendations for setting cultivation parameters
  - ✓ In order to obtain sufficient oxygen exchange, the liquid level height during plate cultivation should not exceed 1-1.2 cm, and the liquid level height during flask cultivation should not exceed 3 mm.
  - ✓ When using Xuri™ Cell Expansion System W25 equipment to culture T cells, it is recommended to set the speed to 8 rpm and the angle to 6 °.
- 2) Recommendations for transduction
  - ✓ If using conventional lentiviral or retroviral transduction, the conventional transduction process can be followed. Alternatively, transduction can be delayed for 1-2 days compared to the serum containing system.
  - ✓ If using nuclear or electroporation, these transduction methods can cause physical damage to the cell membrane. Cells need to absorb or synthesize membrane related lipid substances to repair the cell membrane.

Stay tuned for more updates.

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