AMMS® CIK Cell Expansion Reagent Kit

Product Name

English Name： AMMS®CIK Cell Expansion Reagent Kit

Product Performance

CatalogueNumber： AS-08

Composition of the Kit：

Component Name	Cat. No.	Specification	quantity	Storage conditions	Product Characteristics	Shelf Life
CIK Reagent A	AS08A	500μL	1 piece	-20℃	Freeze - dried powder	24months
CIK Reagent B	AS08B	500μL	1 piece	-20℃	Freeze - dried powder	24months

Product Description

This product is applicable to autologous peripheral blood PBMC or cord blood CBMC, from which CIK cells with a relatively high purity can be obtained through in - vitro activation and amplification. It is for in - vitro research use only.

Instructions for Use

Culture Process：

Day 0	Day 1	Day 4	Day 6	Day 8	Day 11	Day 14 / 15
Separation PBMC	Start activation	First amplification	Second amplification	Third amplification	Fourth amplification	Harvest cells

Notes：

* Preparation of amplification solution: before the first feeding (day4), add one recombinant human IL-2 protein (high efficiency type) to the opened first bottle of culture medium after redissolving.

* Feeding time: there is no abnormality observed under the microscope, the cells grow well, the medium turns yellow, and can start to amplification. If the growth is average or poor, consider reducing the amplification or delaying the amplification.

Seeding CIK primary cells (day 0):

1. Collect blood and separation PBMC, inactivate plasma for future use.

2. Seed the cells (Seed density $1x10^6$ cells/ml) into the culture flask with serum-free medium, with a final volume of 30~50ml. Add CIK reagent A and 1.5~5ml of inactivated plasma (5% of the final volume).

3. Put the culture bottle into the CO2 incubator for culture.

CIK cell activation induced amplification:

Day1: Add CIK reagent B, put into CO_2 incubator for further culture.

Day4: Feed about 100mL, add inactivated plasma 5~ 10mL. It can also be replenished according to the density. The density is between 0.5~ 1 × 106 cells/mL after feeding. (Total volume 150mL)

Day6: Add inactivated plasma about 10~20ml ， evenly transfer the cell suspension to the culture bags and feeding. (Total volume 450ml)

Day8: Feed 550mL.Bacteria detection. (Total volume 1000ml)

Day11: Divide the cell suspension evenly into two culture bags and feed equal volume. (Total volume 2000ml)

Day13: Bacteria, endotoxin and mycoplasma detection.

Day14/15: Harvest cells.

Precautions

Use of the culture medium: Before each replenishment of the culture medium, it should be naturally warmed to room temperature. It is prohibited to place the whole bottle of culture medium in an incubator at 37°C for warming, otherwise it will accelerate the inactivation of cytokines in the replenished culture medium.

Use of the culture bag: When the culture volume is less than 1L, the culture bag needs to be folded before placement. It is recommended to use the models recommended by our company.

Control of cell aggregation: Before loading the cells into the bag, the cells need to be fully dispersed by patting according to the situation of the cell clones. After loading the cells into the bag, the bag should also be patted every day, and the larger cell aggregates observed with the naked eye should be kneaded.

Equipment maintenance: Regularly check the temperature and concentration in the CO2 incubator and replace the filter screen in a timely manner. Regularly maintain and clean the biological safety cabinet.

Environmental monitoring: Regularly replace the primary, medium-efficiency, and high-efficiency filters to ensure the environmental standards of the clean area.

Fix the types and models of experimental consumables: It is necessary to evaluate in advance the impact of changes in models and specifications on the culture effect, such as culture flasks, cell culture bags, etc.

References

1. X. Zhu, W.D. Marcus, W. Xu, H.I. Lee, K. Han, J.O. Egan, J.L. Yovandich, P.R. Rhode, H.C. Wong, Novel human interleukin-15 agonists, J Immunol 183(6) (2009): 3598-3607.

2. M. Chirifu, C. Hayashi, T. Nakamura, S. Toma, T. Shuto, H. Kai, Y. Yamagata, S.J. Davis, S. Ikemizu, Crystal structure of the IL-15-IL-15Ralpha complex, a cytokine-receptor unit presented in trans, Nat Immunol 8(9) (2007)： 1001-1007.