AMMS® NK Cell Culture Kit 2.0

Product Name

English Name:AMMS® NK Cell Culture Kit 2.0

Product Performance

Catalog Number: AS-22

Composition of the Kit:

AMMS® NK Cell Culture Kit. 2.0(Product No:AS22- 1）

Component Name	Cat. No.	Specification	Amount	Storage conditions	Product Characteristics	Shelf Life
NK reagentA-2 .0	AS22- 1A	200μL	1 stick	-20℃	Liquid	18months
NK reagentB-2 .0	AS22- 1B	500μL	1 stick	-20℃	Liquid	18months
NK reagentC-2 .0	AS22- 1C	500μL	1 stick	-20℃	Liquid	18months
NK reagentD-2 .0	AS22- 1D	500μL	1 stick	-20℃	Liquid	18months

AMMS® NK Serum-free Medium(Product No:AS01-2)

Product name	Cat. No.	Specification	Specification	Amount	Product Characteristics	Shelf Life
AMMS® NK Serum-free Medium	AS01-2	1000mL	2 flasks	2~8℃, Protect from light	Liquid	18 months

Product Description

This product is applicable to fresh peripheral blood PBMC, from which NK cells with relatively high purity can be obtained through in vitro activation and amplification. It is for in vitro research use only.

Instructions for Use

Steps	Cultivation time	Use of reagents	Cultivation container	Complete culture medium	Inactivated plasma	Total volume	Remarks
Coating	Day -1	NK reagent A-2.0	175cm^2 Culture flask	/	/	/	Keep coating flask flat at 4℃ overnight.

Seeding	Day 0	NK reagent B-2.0	175cm^2 Culture flask	22.5mL	2.5mL	25mL	Density of seed: 1× 10^6 pcs/mL
foster	Day 3 (1st feed)	NK reagent C-2.0	175cm^2 Culture flask	46.5mL	3.5mL	75mL	Do not disperse cells. Do not touch the cell layer at the bottom of the flask when replenishing the medium.
	Day 5 (2ndfeed)	NK reagent D-2.0	175cm^2 Culture flask	About 166.25mL	8.75mL	250mL	
Bagging	Day7 (3rd feed)	Complete culture medium	Cell culture bag	About 350mL	Remaining plasma	600mL	It can also be replenished according to the density, The density is between 0.6~ 1× 10^6 cell/mL after seed. Pat the culture bag after feeding. Make the cells evenly distributed.
Split bag	Day9 (4th feed)	Complete culture medium	Cell culture bag	Aad to each bag 300 mL	/	1200mL	
	Day11/12 (5thfeed)	Complete culture medium	Cell culture bag	Add to each bag 400 mL	/	2000mL	
Harvest	14 day、15 day	/	Cell culture bag	/	/	2000mL	Harvest cells

Notes：

* The medium should be allowed at room temperature for more than 1h before each use （ Disable the relevant equipment to force rapid rewarming） ，this is the same for subsequent operations .

* Inactivated plasma is calculated at 5% ~ 10% of the culture system. If there are more anticoagulants, it is recommended that the plasma be increased to 7% ~ 12%.

AMMS®NK Cell Culture Kit 2.0 Reference Application Method

Coating Pretreatment of cell activation flask (Day - 1)

Mix 1 vial NK Reagent A-2.0 with 13mL D-PBS into a 175cm2 flask and shake to mix well, keep flat. Or mix 1 vial NKReagent A-2.0 with 9mL D-PBS and add to a 75cm2 flask and shake to mix well, keep flat. Keep in a 4° C freezer overnight. The next day, the coating solution is discarded before the seeding flask.

Seeding in flask Peripheral blood PBMC separation and induction (Day 0)

1 、**Separation of plasma .** Take a small amount of blood sample (about 300 μ L）draw or drip into a flat dish for bacteria detection. Centrifugation at room temperature 15 minutes, take supernatant as plasma.

2 、**Plasma inactivated.** 56℃ inactivated of upper plasm for half an hour, keep in 4℃ for half an hour and take it out.Centrifugation 10 minutes at room temperature, take supernatant standby.

3 、**Separation PBMC.** Mix the equal volume of saline with blood cell precipitation and add to ficoll layer. Keep the layering clear. Centrifugated at room temperature for 25 minutes.

4 、**Wash the cells .** Collect PBMC layer, add saline, mix well. Centrifugation at room temperature for 5 minutes. Wash the cells again .

5 、**Cell counting.** Abandoned supernatant , use a small amount of complete medium to resuspended cells , and absorb a small amount of cell count. Adjust cell density to 1-1.5×10^6 cells/mL.

6、**Seed in flask**. Absorb the coating solution, add cell suspension, NK reagent B-2.0 and 2.5mL inactivated plasma into the culture flask, and the final volume of the culture is about 25mL. Remaining plasma keep at 4℃ for later use .

Note：

* Preparation of complete medium: 1 vial IL-2 is added in each flask of medium is added 1 Branch. The final concentration of IL-2 is 1000IU/mL.

* The time for the coating flask to be taken out of the refrigerator is approximately ten minutes before the cells are added.

Culture The first feeding（Day 3）

1 、Cells are observed under a microscope to determine whether they can be feeded. ①The clone of reaches more than 30% of the bottom area ② The color is yellowish compared to the initial culture medium .（If you can't juage , you can postpone feeding for a day.）

2 、Feeding operation . Add NK reagent C-2 .0 and 3.5 mL of inactivated plasma, add approximately 46.5 mL of complete medium , and the final volume of culture is 75 mL.

Note: * Do not disperse the cells!

The second feeding (Day 5)

3 、Add NK reagent D-2 .0 and 8.75 mL of inactivated plasma, then add approximately 166.25 mL of complete medium to finalize the culture volume to 250 mL.

Note:

* Do not disperse the cells!!

* Cell proliferation is obvious at the beginning of day 5, with more medium and large clumps and more dividing phase morphological cells .

Bagging The third feeding (Day 7)

4、The remaining plasma is added to the culture flask, and then the cell suspension in the flask is transferred to the cell culture bag , finally, replenishing (about 350mL of complete medium , or it can be replenished according to the density, and the density after feeding is within the range of 0.6 ~ 1 × 106 cells/mL), and the final volume of culture is fixed to 600mL.

Note:

* Before bagging , gently pat the cells at the bottom of the flask, if the clone is too large, you can disperse, pay attention to the strength of the dispersing to avoid distributing the clone into a single cell.

* After bagging , the culture bag should be patted regularly to maintain the cell mass at the size of the needle eye observed by the naked eye .

Split bag The Fourth time feeding (Day 9)

① Prepare another bottle of NK complete medium . ② Divide half of the cell suspension in the bag into a new culture bag, and then add another 300mL of complete culture base to each bag. (Final culture volume is 1200 mL) .

The Fifth time feeding (Day 11/12)

Aliquot the remaining approximately 800 mL of complete medium into 2 bags with a final volume of approximately 1000 mL each.

Test On day 13 of cultivation

a small amount of cell suspension is taken from the bag with a 5mL syringe for bacteria, endotoxin, mycoplasma detection .

Harvest

Normally, harvest 1,000 mL of cell suspension on days 14 and 15. If required by the experiment, it can be earlier or delayed accordingly.

If a larger culture volume is required, the NK cell culture time can be extended (it can be extended to 21 days, and the AMMS® NK Serum-Free Medium and IL-2 need to be purchased additionally). Then, continue to add the complete NK medium. After adding the medium, the cell density should not be lower than 1×10^{6} cells/mL.

Precautions

1、**Blood Sample Requirements:**

① The number of peripheral blood PBMC should be more than 2.5×10^7 cells (it is recommended to collect about 50 mL of blood using a sodium heparin vacuum blood collection tube). It is advisable to carry out the operation within 4 hours after blood collection, and lymphocyte subset analysis is recommended. It is not recommended to use cryopreserved peripheral blood.

2、**Seeding Density in the Culture Flask:** The recommended initial seeding density of PBMC in the culture flask is $1\text{-}1.5\times10^6$ cells/mL. If the sample condition is poor, the seeding density can be appropriately increased to 2×10^6 cells/mL.

3、Medium Replenishment Density: The density before medium replenishment is generally $1.5\text{-}2\times10^6$ cells/mL; the density after medium replenishment is generally $0.6\text{-}1\times10^6$ cells/mL, and it should not be lower than 0.6×10^6 cells/mL.

4、Use of the Culture Medium:

① Before each medium replenishment, the culture medium should be naturally warmed to room temperature.

② It is prohibited to place the whole bottle of culture medium in a 37℃ incubator for warming, otherwise it will accelerate the inactivation of cytokines in the replenished culture medium.

③ The prepared amplification medium (containing IL-2) has a relatively short shelf life. It is recommended to use it up within about one week, especially in the early activation stage (the first 7 days).

5、Proper Handling and Storage of Plasma: Refer to the instruction manual for details. The plasma after centrifugation should be ensured to be clear.

6、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.

7、Master the Timing of Medium Replenishment Flexibly: When the cell amplification status is not ideal, the medium replenishment time can be postponed, but try not to adjust the volume of the medium replenishment, especially pay attention to the timing of the first medium replenishment. The volume of the medium replenishment after bagging can be adjusted according to the culture time.

8、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.

9、Coating Time: After coating with Factor A, it needs to be placed flat at 4℃ overnight. (In case of emergency, try coating at 37℃ for 2 hours.)

10、Do not Shake the Culture Flask Casually in the Early Stage of Culture: Otherwise, the activated cell clones are likely to float up, reducing the activation effect of the coating factor on the cell clones.

11、Use of Factors: To reduce the loss of factors due to wall adhesion, it is recommended to perform centrifugation before use. Put the vial containing the factor into a 50 mL centrifuge tube and centrifuge at 1000 rpm for 1-2 minutes.

12、Equipment Maintenance: Regularly check the temperature and concentration in the CO_2 incubator and replace the filter screen in a timely manner. Regularly maintain and clean the biological safety cabinet.

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

14、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 175 cm^2 culture flasks, cell culture bags, etc.

References

1. Garnet Suck, Mickey Boon Chai Koh, Emerging natural killer cell immunotherapies: large-scale ex vivo production of highly potent anticancer effectors, Hematol Oncol Stem Cel Ther 2010; 3(3): 135-142 .

2. Malgorzata Grudzien and Andrzej Rapak,Review Article Effect of Natural Compounds on NK Cell Activation Laboratory of Tumor Molecular Immunobiology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw 53-114, Poland.