AMMS® NK Cell Culture Kit 2.0

Catalog Number: AS-22

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

Component Descriptions						
Component Name	**Cat. No.**	**Specification**	**Amount**	**Storage**	**Product characteristics**	**Shelf Life**
NK reagent A-2.0	AS22- 1A	200μL	1 stick	-20℃	Liquid	18 Months
NK reagent B-2.0	AS22- 1B	500μL	1 stick	-20℃	Liquid	18 Months
NK reagent C-2.0	AS22- 1C	500μL	1 stick	-20℃	Liquid	18 Months
NK reagent D-2.0	AS22- 1D	500μL	1 stick	-20℃	Liquid	18 Months

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

Product name	**Cat. No.**	**Specification**	**Amount**	**Storage**	**Product characteristics**	**Shelf Life**
AMMS® NK Serum-free Medium	AS01-2	1000mL	2 flasks	2~8℃	Liquid	18 Months

Description

This product is suitable for autologous peripheral blood or umbilical cord blood PBMC, through in vitro activation and amplification to obtain higher purity of NK cells. Only for in vitro research use.

Product 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: 2× 10^6 pcs/mL
Culture	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 (2nd feed)	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	
	Day 11/12 (5th feed)	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 175cm^2 flask and shake to mix well, keep flat. Or mix 1 vial NK Reagent A-2.0 with 9mL D-PBS and add to a 75cm^2 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 300μL）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 (for umbilical cord blood, 8 minutes is recommended). 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 2× 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)

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× 10^6 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.

AMMS®NK Cell Culture Kit 2.0 Usage Considerations

1、Blood sample requirements：

①Peripheral blood PBMC>3× 10^7cells (recommended blood collection volume of about 50mL, vacuum blood collection tube with heparin sodium blood), it is recommended to start the process within 4 hours after blood collection and run lymphocyte subset analysis . Cryopreserved peripheral blood is not recommended.

②The recommended volume of cord blood (without anticoagulant) is >50 mL, it is recommended to withdraw at least half (approximately 14 mL) of the anticoagulant from the blood collection bag, and no more than 14% (or PBMC>3× 10^7 cells of the other container)for another container. Start within 12 hours after blood collection.

Note: *The amount of mononuclear cells that can be extracted from 50mL of umbilical cord blood without containing anticoagulant is generally about 5~8× 10^7. Use this as a reference.

2、Seed flask density: The starting cell density of PBMC is recommended to be 2× 10^6 cells/mL in flask, and cord blood can appropriately increase the initial cell density.

3、Feeding density: the density before feeding is generally 1.5~2 × 10^6 cells/mL; The density after feeding is generally 0.6 ~ 1 × 10^6 cells/mL, not less than 0.6 × 10^6 cells/mL.

4、Use of media:

(1) Before each replenishment, the medium needs to be naturally rewarmed at room temperature.

(2) Do not rewarm the whole vial medium in a 37°C incubator, otherwise it will accelerate the inactivation of cytokines in the feeding medium.

(3) The expansion medium (containing IL-2) has a short aging period and is not recommended for more than one week, especially in the pre-activation period (the first 7 days).

5、Correct handling and preservation of plasma: See instructions for details. The plasma after centrifugation should be clarified, and the dilution effect of the anticoagulant on the plasma should be considered when adding plasma.

6、Use of culture bags: When the culture volume is less than 1L, the culture bag needs to be folded and placed. It is recommended to use our recommended size.

7、Flexible timing of feeding: When the cell expansion status is not ideal, the feeding time can be postponed, but try not to adjust the volume of feeding, especially pay attention to the timing of the first feeding. The volume of feeding after bagging can be adjusted according to the culture time.

8、Control cell clumping: Before the cells are bagged, the cells need to be thoroughly patted according to the situation of the clonal cluster. After bagging, it is also necessary to pat the bag every day and rub the larger cell mass which can be observed by naked eye.

9、Coating time: After factor A coating, it should be laid flat at 4°C overnight. (In case of emergency, try 37°C coating for 2 hours)

10、Do not shake the flask at the beginning of culture: otherwise the activated clone will float up easily, and reduce the activation of the cell mass by the coating factor.

11、Equipment maintenance: regularly check the temperature and concentration of the CO_2 incubator and replace the filter in time. Regular maintenance and cleaning of the biosafety cabinet.

12、Environmental monitoring: regularly replace the primary, medium and high-efficiency filters to ensure the environmental standards of clean areas.

13、Fixed experimental consumables type and model: It is necessary to evaluate the impact of changing the specification on the culture effect in advance, such as 175 flasks, cell culture bags, etc.

Product Use

For research and manufacturing use