AMMS®NK Cell Culture Kit 3.0

Product Name

English Name:AMMS®NK Cell Culture Kit 3.0

Product Performance

Catalog Number: AS-25

Composition of the Kit：

AMMS® NK Cell Culture Kit 3.0-A/D (Catalog number: AS25-1A/D)

Component Name	Cat. No.	Specification	Amount	Storage conditions	Product Characteristics	Shelf Life
NK reagent A-2.0	AS22-1A	200μL	1 stick	-20℃	Liquid	18months
NK reagent B-2.0	AS22-1B	500μL	1 stick	-20℃	Liquid	18months
NK activation additive 1	AS25-1C	1000μL	1 stick	-20℃	Liquid	18months
NK activation additive 2	AS25-1D	100μL	1 stick	-20℃	Liquid	18months

AMMS® NK cell culture kit 3.0-E/G (Catalog number: AS25-1E/G)

Component Name	Cat. No.	Specification	Amount	Storage conditions	Product Characteristics	Shelf Life
NK amplification additive 1	AS25-1E	1000μL	2 stick	2-8℃	liquid	18months
NK amplification additive 2	AS25-1G	50μg	1 stick	2-8℃	dry powder	18months
Recombinant human IL-2 protein (highly	TL-402-0100F	1×10^6IU	2 stick	2-8℃	dry powder	18months

AMMS® NK activated medium (Catalog number: AS25-2)

Product name	Cat. No.	Specification	Specification	Amount	Product Characteristics	Shelf Life
AMMS® NK activated culture medium	AS25-2	100mL	1 bottle	2~8℃, Protect from light	liquid	18 months

AMMS® NK expanded culture medium (Catalog number: AS25-3)

Product name	Cat. No.	Specification	Specification	Amount	Product Characteristics	Shelf Life
AMMS® NK expanded culture medium	AS25-3	1000mL	2 bottles	2~8℃, Protect from light	liquid	18 months

Product Description

This product is suitable for fresh or cryopreserved peripheral blood PBMC and umbilical cord blood CBMC, and high purity NK cells are obtained by in vitro activation and amplification. It is only for in vitro research use.

Instructions for Use

Steps	Cultivation time	Use of reagents	Cultivation container	Complete culture medium	HPL or inactivated plasma	bulk volume	Remarks
Coating	-1 day	NK reagent A-2.0	75cm2 culture flask	/	/	/	Place the bottle in a bag for 4℃ overnight
Seeding	Zero days	NK reagent B-2.0 NK was fully activated in the culture medium	75cm2 culture flask	23.75mL	1.25mL	25mL	The density of the bottle was 1x106 /mL
foster	Three days (First fluid replacement)	NK was fully activated in the culture medium	75cm2 culture flask	23.75mL	1.25mL	50mL	Do not blow the cells, and do not touch the cell layer at the bottom of the bottle when adding culture medium
	Six days (2nd fluid replacement)	NK was fully activated in the culture medium	75cm2 culture flask	23.75mL	1.25mL	75mL	
Change the bottle	Seven days (3rd fluid replacement)	NK complete activation medium NK complete expansion medium	175cm2 culture flask	71.25mL	3.75mL	150mL	Add NK cell complete medium (count cells and NK cell purity test as needed) to the cell suspension at a volume ratio of 1:1 with fresh medium, and add 5%HPL (5% autologous plasma).
bagging-off	Nine days (4th fluid replacement)	NK fully expanded culture medium	Cell culture bags	142.5mL	7.5mL	300mL	
	11 days (5th fluid replacement)	NK fully expanded culture medium	Cell culture bags	285mL	15mL	600mL	
	13 days (6th fluid replacement)	NK fully expanded culture medium	Cell culture bags	600mL	/	1200mL	/

Bag it	15 days (7th fluid replacement)	NK fully expanded culture medium	Cell culture bags	900mL	/	2100mL	/
gather in the crops	17 / 18 days	/	Cell culture bags	/	/	2100mL	collecting cell

Note:

* The culture medium should be left at room temperature for more than 1h before each use (do not use related equipment to force rapid re-temperature), and the subsequent operation is the same.

* If the umbilical blood volume exceeds 80mL, autologous plasma can be added; if the blood volume is less than 80mL, HPL can be used to replace autologous plasma.

* Configuration of fully activated culture medium: add 1 bottle of NK activation culture medium with 1 bottle of NK activation additive 1 and 1 bottle of NK activation additive 2.

* Configuration of fully amplified culture medium: 1 bottle of NK amplification culture medium was added with 1 bottle of NK amplification additive 1, 25ng/mL of NK amplification additive 2 and 1 bottle of recombinant human IL-2 protein, and the final concentration of IL-2 was 1000IU/mL

AMMS®NK kit 3.0 reference operating method:

Coating Pretreatment of cell activation flask (Day - 1)

1 ml of NK reagent A-2.0 and 9mL D-PBS were mixed, added to a 75cm2 culture bottle, spread evenly on the flat side, and left overnight in a 4℃ refrigerator. The next day, the coating solution was removed before planting the bottle.

PBMC or CBMC isolation and induction in vials (Day 0)

1、Separate plasma. Take a small amount of blood sample (about 300 μ L) and draw or drop it into a petri dish for bacterial detection. Centrifuge at room temperature for 15 minutes, and take the supernatant as plasma.

2、Plasma inactivation. The upper plasma was inactivated for half an hour at 56℃, placed in a 4℃ refrigerator for half an hour, taken out and centrifuged at room temperature for 10 minutes, and the supernatant was taken for later use.

3、Separate PBMC or CBMC. Mix equal volume of normal saline with the blood cell sediment, add to the Ficoll layer to keep the stratification clear, and centrifuge at room temperature for 25 minutes.

4、Wash the cells. Aspirate the PBMC or CBMC layer, add normal saline and blow to mix, centrifuge at room temperature for 8 minutes. Wash the cells again.

5、Cell counting. Discard the supernatant, resuspend the cells in a small amount of fully activated medium, and aspirate a small amount of cells for counting. Adjust the cell density to $1x10^6$ cells/mL.

6、Vial. Absorb the coating solution, add NK reagent B-2.0,HPL1.25mL or inactivated plasma 1.25mL to the cell suspension, transfer into the culture bottle, and the final volume of culture is about 25mL. The remaining plasma 4℃ is sealed for storage.

Note: * The incubation bottle should be removed from the refrigerator about 10min before the cells are added.

Cultivation of the first fluid replacement (day 3)

1、Rehydration operation. Under the microscope, the cell mass at the bottom of the bottle reached more than 20% of the area at the bottom of the bottle. NK cells were supplemented with 25mL complete activated culture medium and 5%HPL (5% autologous plasma) according to the bottle, and the final volume of the culture was 50mL.

Note: * Do not blow the cell!!!

Second fluid replacement (day 6)

2、Rehydration operation. Under the microscope, the cells were observed and the cell proliferation was obvious, and the medium was supplemented with NK cells at 25mL/ bottle to completely activate the culture, and 5%HPL (5% autologous plasma) was added. The final volume of the culture was 75mL.

Note: * Do not blow the cell!!!

Change of bottle Third fluid replacement (day 7)

3、Replenishment and bottle change procedure. Add NK cell complete medium at a volume ratio of 1:1 to the cell suspension, transferring the cell suspension from T75 flasks to T175 flasks. Supplement with NK cell complete expansion medium (75mL per flask) when needed, and add 5%HPL (5% autologous plasma). The final culture volume is 150 mL.

Bagging for the fourth fluid replacement (day 9)

4、Rehydration procedure. Transfer the cell suspension from the culture flask to the cell culture bag, and add NK cell complete medium at a volume ratio of 1:1 with fresh culture medium. Add 150 mL of NK cell complete expansion medium and 5%HPL (5% autologous plasma). After rehydration, the final volume is 300 mL. Perform cell counting and NK cell purity testing as needed, ensuring that the density after rehydration is no less than 1×10^6/mL.

Fifth fluid replacement (day 11)

5、Rehydration procedure. Add NK cell complete medium at a volume ratio of 1:1 to the cell suspension and fresh culture medium. Add 300 mL of NK cell complete expansion medium and 5%HPL (5%

autologous plasma). After rehydration, the final volume is 600 mL. Count cells and check NK cell purity as needed. The density after rehydration should not be less than 1×10^6/mL.

Sixth fluid replacement (day 13)

6、Rehydration operation. Add NK cell complete medium and 600mLNK cell complete expansion medium according to the volume ratio of 1:1 between the cell suspension and fresh culture medium, and the final volume after rehydration is 1200mL. Count cells and check NK cell purity as needed, and ensure that the density after rehydration is no less than 1×10^6/mL.

Bagging for the seventh fluid replacement (day 15)

7、The operation of bag separation and fluid replenishment. Half of the cell suspension in the culture bag was added to a new culture bag, and then 450mL complete medium was added to each bag. The final volume of the culture was 2100mL. Cell counting and NK cell purity detection were carried out as required, and the density after fluid replenishment was not lower than 1×10^6/mL.

Harvest (17 / 18 days)

8、Under normal circumstances, 1050mL cell suspension was harvested on days 17 and 18. If necessary, the harvest time could be advanced or delayed accordingly.

9、If more culture volume is required, the NK culture time can be extended (the culture can be extended to 21 days, and additional AMMS® NK amplification culture medium and AMMS® NK cell culture kit 3.0-E/G should be purchased), and NK complete amplification culture medium should be added continuously. After replenishment, the density should not be lower than 1×10^6 cells/mL.

Precautions

1、**Sample requirements:**

① If the blood volume of umbilical cord blood (including anticoagulant) is less than 80mL or the expected NK harvest is more, it is recommended to use HPL instead of autologous plasma. The cell proliferation rate of HPL is about 1-2 times higher than that of autologous plasma.

② The operation should be completed within 12 hours after blood collection.

③ It is recommended that the freezing density of frozen samples be 2-4 x 10 7/mL, and the viability rate after recovery should not be lower than 80%. HPL should be used instead of autologous plasma during the cultivation of umbilical cord blood samples. Autologous plasma should be used for peripheral blood samples (heparin sodium anticoagulant), and HPL can also be used instead of autologous plasma.

2、Vessel density: the initial cell density of the bottle is recommended to be 1x10 6 cells/mL. When there are more red blood cells, the fluorescence counting or red blood cell lysis counting should be selected to avoid affecting the bottle density of the cell.

3、Fluid density: the density after fluid replacement should not be lower than 1x10 ^{6}/mL.

4、Use of culture medium:

① The culture medium should be naturally rewarmed at room temperature before each infusion.

② Do not put the whole bottle of culture medium into the 37℃ incubator for re-temperature, otherwise it will accelerate the inactivation of cytokines in the supplemented culture medium.
③ The prepared complete medium has a short shelf life, and it is recommended to use it up in about one week.

5、Proper handling and preservation of plasma: see instructions for details. Centrifuged plasma should be clear.

6、 Use of culture bags: When the culture volume is less than 1L, the culture bags need to be folded before being placed. It is recommended to use the recommended model of our company.

7、 Incubation time: A, the factor should be incubated for 4℃ overnight in a flat bed. (In case of emergency, try to incubate 37℃ for 2 hours)

8、Do not shake the culture bottle at will during the initial stage of culture: otherwise, the activated clone group is easy to float up, and the coating factor will reduce the activation of the cell group.

9、Use of factors: In order to reduce the loss of factors hanging on the wall, it is recommended to perform centrifugation before use. Place the vial containing factors into a 50mL centrifuge tube and centrifuge at 1000rpm for 1-2min.

10、 Equipment maintenance: check the temperature and concentration of CO2 incubator regularly, and replace the filter in time. Regular maintenance and cleaning of biological safety cabinet.

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

12、 **Fixed types and models of experimental consumables:** the impact of changing models and specifications on cultivation effects should be evaluated in advance, such as 75cm2 culture bottles and cell culture bags.

References

1.Umbilical Cord Blood Transplantation: Connecting Its Origin to Its Future.2023 Mar 3;12(2):55-71.
2.Natural Killer Cell Expansion and Cytotoxicity Differ Depending on the Culture Medium Used.2022 Nov 1;42(6):638-649.