

SUPERCULTURE Natural Killer Cells Induction Culture Kit 2.0

Cat#: 6813531/6813532/6813533

[Product Name]

Natural Killer Cells Induction Culture Kit 2.0

[Product Description]

Natural Killer Cells Induction Culture Kit 2.0 is a GMP-grade in vitro induced expansion culture kit suitable for natural killer feeder-free cells, including NK Cell Stimulator, NK Cell Activator and N500 Serum-Free Medium for NK cells. This product has stable inter-batch quality and is used to expand culture NK cells from human peripheral blood mononuclear cells (PBMCs).

[Model & Specification]

Product Name	Cat. No.	Size	Component Name	Component Size	Quantity
Natural Killer Cells Induction Culture Kit 2.0	6813531	3 L/kit	NK Cell Stimulator	150 µL	1 pcs
			NK Cell Activator	500 µL	3 pcs
			N500 Serum-Free Medium for NK cells	1 L	3 bottle
	6813532	2 L/kit	NK Cell Stimulator	100 µL	1 pcs
			NK Cell Activator	500 µL	2 pcs
			N500 Serum-Free Medium for NK cells	1 L	2 bottle
	6813533	1 L/kit	NK Cell Stimulator	50 µL	1 pcs
			NK Cell Activator	500 µL	1 pcs
			N500 Serum-Free Medium for NK cells	1 L	1 bottle

[Storage Conditions and Validity Period]

NK Cell Stimulator, NK Cell Activator: Store at -15°C~ -25°C, valid for one year.

Serum-Free Medium for NK cells: Keep away from light at 2~8°C, valid for one year.

[Directions for Use]

Preparation

1. Medium formulation: Add 1 vial of 500 µL NK Cell Activator to every 1 L of Serum-Free Medium for NK cells to prepare the Amplification Medium for NK cells. Amplification Medium for NK cells is valid for 3 weeks.

2. Heat-inactivated autologous plasma: Separate the blood collected to obtain plasma, perform heat treatment on the plasma at 56°C for 30 min, centrifuge 1000 g for 10 min, take the supernatant to prepare the heat-inactivated autologous plasma.

Sample Requirements

Fresh peripheral blood mononuclear cells (PBMCs) with viability ≥ 90%.

NK cell culture (take fresh PBMCs, 1 L system as an example):

3. On Day 0, inoculate separated 1.5×10^7 PBMCs into a T75 flask using 5 mL **Amplification Medium for NK cells** containing 10% heat-inactivated autologous plasma (inoculum density recommended as 3×10^6 cells/mL), and add 50 µL NK Cell Stimulator, culture in a 37°C incubator containing 5% CO₂.

4. On Day 3, supplement 10 mL of fresh **Amplification Medium for NK cells** (containing 10% heat-inactivated autologous plasma).

5. From Day 5, take samples for counting every other day, and supplement fresh **Amplification Medium for NK cells** (containing 5% heat-inactivated autologous plasma) to adjust the NK cell density to $0.8 \sim 1.0 \times 10^6$ cells/mL. Select a larger flask or transfer into the cell culture bag according to the volume of cell culture suspension. The maximum culture volume of T75 flask is 40 mL and

of T175 flask is 200 mL. When the medium volume exceeds 200 mL, transfer into the cell culture bag for culture.

6. After Day 7, the heat-inactivated autologous plasma content in the fresh Amplification Medium for NK cells supplemented could be reduced to 1%.

7. Test of cell proliferation and cell surface markers.

Rapid fluid supplementation procedure (for reference only):

Days/d	Original Medium /mL	Medium Added/mL	Plasma Added/mL	Total Medium Volume/mL	Reagent Added	Culture Flask/Culture Bag
0	0	4.5	0.5	5	50 μ L Stimulator	T75 culture flask
3	5	9	1	15		T75 culture flask
5	15	28.5	1.5	45		T175 culture flask
7	45	89.1	0.9	135		T175 culture flask
9	135	267.3	2.7	405		Transfer into culture bag
11	405	589	6	1000		

[Notes]

- Avoid repeated freezing and thawing of this product, and pay attention to operate aseptically during use.
- On Day 0, add NK Cell Stimulator at 1% of the medium volume (including plasma volume).
- The cell inoculum density on Day 0 can fall within the range of $2.5\sim 3.5 \times 10^6$ cells/mL.
- The blood collection volume can be estimated based on 1×10^6 PBMCs per milliliter of peripheral blood.
- During blood collection, heparin anticoagulant can be used, but EDTA anticoagulant cannot be used.
- Before use, the medium should be equilibrated at room temperature, or the expected amount on the day of removal should be prewarmed to 37°C. Do not place the whole bottle of medium at 37°C for repeated prewarming.
- If the temperature of the medium is too low or the cell density is too high, flocculated cells may appear, and the cell viability is reduced.
- Cell passage shall be carried out gently to avoid mechanical damages to cells.
- 3 L system 6813531 is applicable to $3.75\sim 5.25 \times 10^7$ PBMCs; 2 L system 6813532 is applicable to $2.5\sim 3.5 \times 10^7$ PBMCs; 1 L system 6813533 is applicable to $1.25\sim 1.75 \times 10^7$ PBMCs.
- For samples with abnormal plasma, Cell Culture Supplemental Mix can be substituted for autologous plasma.

[Description of Product Symbol]

Product Symbol	Description	Product Symbol	Description
	Catalogue number		Batch code
	Date of manufacture		Temperature limit
	Use-by date		Consult instructions for use
	Manufacturer		Keep away from sunlight

[Related Products]

Product Name	Cat. No.	Size
Natural Killer Cells Induction Culture Kit	6813521	3 L/kit
	6813522	2 L/kit
	6813523	1 L/kit
N500 Serum-Free Medium for NK Cells	6113031	1000 mL
NK Cell Activator	XC0032A	500 µL
NK Cell Stimulator	XC0031A	500 µL
Cell Culture Supplemental Mix	6122012	25 mL
	6122011	250 mL
Density Reagent (A)	7912011	250 mL
Human Lymphocyte Separation Medium	7111011	100 mL
Lymphocyte Separation Tube for Human Peripheral Blood	7922021	50 mL × 25 vials
Human Lymphocyte Separation Kit (GMP)	7825121	60 tests
Human Lymphocyte Separation Kit	7125121	60 tests
Cell Culture Bags	6071011	640 cm ² , 10 bags/package
	6071012	640 cm ² , 10 bags/package × 10, 100 bags/carton

[Instruction Revision Date]

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[Company Information]**Manufacturer and after-sales service unit Name:**

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