ArtMedia® Human NK Cell Serum-Free Expansion Kit

Please read the manual carefully before use

Cat. No. MK101

Version No. Version 1.0

Storage: NK Serum-Free Medium: at 2–8°C for 1 year. Other Components: at -18°C or below for 1 year, protected from light, avoid repeated freeze-thaw cycles.

Description

The *ArtMedia*® Human NK Cell Serum-Free Expansion Kit is a serum-free, xeno-free, and pure cytokine based in vitro induction and expansion kit designed for NK cell. It includes NK serum-free medium, NK activation factor, NK Cytokine I, II, and III. It is suitable for the culture and expansion of NK cells derived from fresh or cryopreserved peripheral blood or umbilical cord blood.

Features

- Serum-free, xeno-free, and chemically defined
- Pure cytokine formulation; no feeder cells required
- Contains phenol red; no antibiotics
- Rapid expansion, high viability, high purity, and potent cytotoxicity
- Manufactured and managed under GMP standards

Kit Contents

Component	MK101-01 (1 L)	MK101-02 (2 L)	Storage
NK Serum-Free Medium	1000 ml	1000 ml×2	2-8°C
NK Activation Factor	1 vial，100 µl/vial	2 vial，100 µl/vial	at -18°C or below, protected from light, avoid repeated freeze-thaw cycles
NK Cytokine I	1 vial，100 µl/vial	2 vial，100 µl/vial	
NK Cytokine II	1 vial, dry powder	2 vial, dry powder	
NK Cytokine III	1 vial，1 ml/vial	2 vial，1 ml/vial	

Protocol (Peripheral Blood Mononuclear Cells (PBMCs) as an Example)

Self-prepared

Component	Catalog
Human Peripheral Blood Lymphocyte Separation Solution	TransGen , Cat. FB102-02
PBS (1×)	TransGen , Cat. FG701-01
25 cm² Culture Flask	TransGen , Cat. CFE-F4025-01
175 cm² Culture Flask	TransGen , Cat. CFE-F4175-01
2 L Cell Culture Bag	
2.5 L Cell Culture Bag	

PBMC Isolation

1) Autologous plasma preparation: Centrifuge anti-coagulated blood at 650×g for 15 min at room temperature. Collect the upper yellow plasma layer (lower layer is containing blood cells for PBMC isolation). Heat-inactivate at 56°C for 30 min. Centrifuge at 900×g for 10 min. Freeze supernatant at -20°C for 15 min. Then centrifuge again at 900×g for 10 min, collect the supernatant, and store at 4°C for up to 2 weeks. If the extracted plasma is not used immediately, it should be stored at -20°C for long-term preservation.

Note: Avoid using EDTA-anticoagulated blood samples, as EDTA can significantly impair the activation and proliferation of NK cells. Heparin-anticoagulated blood is recommended. Collected blood samples should preferably be processed within 12 hours.

2) PBMC isolation: Dilute the lower red pellet (from step 1) with an equal volume of PBS (equal to the original blood volume). Slowly layer diluted blood onto lymphocyte separation medium (TransGen, Cat: FB102) (the volume of the separation solution should be equal to that of the original blood), ensuring a moderate layering speed (refer to the operating procedure of TransGen, Cat: FB102 for guidance). Centrifuge at 800×g for 20 min at room temperature (slow acceleration/deceleration). Collect the white buffy coat carefully into a new centrifuge tube, mix well with PBS (5–10 times the volume of the buffy coat solution), centrifuge at 300×g for 10 min. Discard the supernatant, wash the pellet by resuspending it with PBS again (5–10 times the volume of the buffy coat solution), perform cell counting, and centrifuge at 300×g for 10 min. Discard the supernatant, and the cells are ready for subsequent use.

1 L Culture System (for 20–30 ml Blood Samples)

1) Pre-treatment of culture flasks (Day -1 or Day 0): Mix 5 ml PBS with 1 vial of **NK activation factor**. Invert to mix, then add to a T25 flask. Swirl to evenly distribute the solution. Incubate at 2-8°C overnight or in 37°C incubator for 2 h.
Note: Coated flasks can be stored at 2–8°C for up to 3 days.

2) **NK activation medium 1 preparation**: Add 12.5 µl **NK cytokine** I to 10 ml **NK serum-free medium**. Mix well.

3) Cell seeding (Day 0): Warm coated T25 flasks to room temperature for 20–30 min. Discard coating solution. Add **9 ml NK activation medium 1**, **1 ml heat-inactivated autologous plasma**, and PBMC (Recommended density: 1.5-2.5 × 10^6 cells/ml; 2.0-3.0 × 10^6 cells/ml for cryopreserved PBMC). Swirl gently and incubate at 37°C, 5% CO_2 .

4) **Preparation of complete NK medium**: Dissolve 1 vial of **NK cytokine II** to 1ml **NK serum-free medium**, then add to **NK serum-free medium** at a ratio of 1:1000. Mix thoroughly.
Optional: Antibiotics (e.g. penicillin-streptomycin) may be added based on experimental requirements.

5) **Preparation of NK activation medium 2**: Add the remaining **NK cytokine I to 60 ml of complete NK medium**. Mix thoroughly.

6) Medium supplementation (Day 3): Slowly add **18 ml of NK activation medium 2 and 2 ml of heat-inactivated autologous plasma** along the sidewall of the culture flask. Ensure the total medium volume per flask reaches 30 ml.
Note: Avoid touching the bottom of the flask. Do not pipette the cells directly. Minimize cell counting, observation, or other handling during the initial growth phase before Day 5.

7) Flask expansion (Day 5): Gently resuspend and transfer all cells from the T25 flask to a T175 flask. Supplement each T175 flask with **38 ml of NK activation medium 2 and 2 ml of heat-inactivated autologous plasma**.

8) **Preparation of NK expansion medium**: Add **NK cytokine III** to the remaining **complete NK medium** at a ratio of 1:1000. Mix thoroughly.

9) Medium supplementation (Day 7): Add 130 ml of **NK expansion medium** and 1.3 ml of **heat-inactivated autologous plasma** to the T175 flask. If the plasma volume is insufficient, add the remaining amount entirely.

10) Transfer to culture bag (Day 9): Resuspend all cells from the T175 flask and transfer to a culture bag. Supplement with 350 ml **NK expansion medium**.
Note: If the total volume in the culture bag is <500 ml, fold the bag for proper placement. Gently shake the bag daily and dissociate cell clumps by tapping to prevent excessive aggregation caused by rapid cell proliferation.

11) Medium supplementation (Day 11): Supplement with **450 ml of NK expansion medium** (adjust based on cell growth status). Quality control: Perform microbial testing for bacteria, fungi, mycoplasma, and endotoxin at this stage.

12) Harvest (Days 14-16): Harvest cells based on their growth status.

Reference Schedule (1L system)

Time	Reagent	Cell Density (10^6/ml)	Container	Add	Total	Supplemented plasma volume	Plasma supplementation ratio
D0	NK Activation Medium 1	1.5-2.5	T25	10 ml	10 ml	1 ml	10%
D3	NK Activation Medium 2	1.5-2.5	T25	20 ml	30 ml	2 ml	10%
D5	NK Activation Medium 2	0.5-1	T175	40 ml	70 ml	2 ml	5%
D7	NK Expansion Medium	0.5-1	T175	130 ml	200 ml	1.3 ml	1%
D9	NK Expansion Medium	0.5-1	2 L Cell Culture Bag	350 ml	550 ml	0 ml	0%
D11	NK Expansion Medium	0.5-1	2 L Cell Culture Bag	450 ml	1000 ml	0 ml	0%
D14-D16	Harvested Cell（1000 ml）						

2 L Culture System (for 50 ml Blood Samples)

1) Pre-treatment of culture flasks (Day -1 or Day 0): Mix 10 ml PBS with 2 vials of **NK activation factor**. Invert to mix, then add to two T25 flasks (5 ml per flask). Swirl to evenly distribute the solution. Incubate at 2-8°C overnight or in 37°C incubator for 2 h.

Note: Coated flasks can be stored at 2–8°C for up to 3 days.

2) **NK activation medium 1 preparation**: Add 25 µl **NK cytokine** I to 20 ml **NK serum-free medium**. Mix well.

3) Cell seeding (Day 0): Warm coated T25 flasks to room temperature for 20–30 min. Discard coating solution. Add **9 ml NK activation medium 1**, **1 ml heat-inactivated autologous plasma**, and PBMC (Recommended density: 1.5-2.5 × 10^6 cells/ml; 2.0-3.0 × 10^6 cells/ml for cryopreserved PBMC) to each T25 flask. Swirl gently and incubate at 37°C, 5% CO_2 .

4) **Preparation of complete NK medium**: Dissolve 1 vial of **NK cytokine II** to 1ml **NK serum-free medium**, then add to NK serum-free medium at a ratio of 1:1000. Mix thoroughly.

Optional: Antibiotics (e.g. penicillin-streptomycin) may be added based on experimental requirements.

5) **Preparation of NK activation medium 2**: Add the remaining **NK cytokine I to 140 ml of complete NK medium**. Mix thoroughly.

6) Medium supplementation (Day 3): For each culture flask, slowly add **18 ml of NK activation medium 2 and 2 ml of heat-inactivated autologous plasma** along the sidewall of the culture flask. Ensure the total medium volume per flask reaches 30 ml.

Note: Avoid touching the bottom of the flask. Do not pipette the cells directly. Minimize cell counting, observation, or other handling during the initial growth phase before Day 5.

7) Flask expansion (Day 5): Gently resuspend and transfer all cells from both T25 flasks to one T175 flask. Supplement with **95 ml of NK activation medium 2 and 5 ml of heat-inactivated autologous plasma**.

8) **Preparation of NK expansion medium**: Add **NK cytokine III** to the remaining **complete NK medium** at a ratio of 1:1000. Mix thoroughly.

9) Transfer to culture bag (Day 7): Resuspend all cells from the T175 flask and transfer to a culture bag. Supplement with 340 ml **NK expansion medium** and 3.4 ml of **heat-inactivated autologous plasma**. If the plasma volume is insufficient, add the remaining amount entirely.

Note: If the total volume in the culture bag is <500 ml, fold the bag for proper placement. Gently shake the bag daily and dissociate cell clumps by tapping to prevent excessive aggregation caused by rapid cell proliferation.

10) Medium supplementation (Day 9): Add 700 ml of **NK expansion medium** to the culture bag. The cell culture bag is now fully expanded.

11) Medium supplementation (Day 11): Supplement with **800 ml of NK expansion medium** (adjust based on cell growth status). Quality control: Perform microbial testing for bacteria, fungi, mycoplasma, and endotoxin at this stage.

12) Harvest (Days 14-16): Harvest cells based on their growth status.

Reference Schedule (2L system)

Time	Reagent	Cell Density (10^6/ml)	Container	Add	Total	Supplemented plasma volume	Plasma supplementation ratio
D0	NK Activation Medium 1	1.5-2.5	T25×2 bottles	10 ml×2	10 ml×2	2 ml	10%
D3	NK Activation Medium 2	1.5-2.5	T25×2 bottles	20 ml×2	30 ml×2	4 ml	10%
D5	NK Activation Medium 2	0.5-1	T175	100 ml	160 ml	5 ml	5%
D7	NK Expansion Medium	0.5-1	2.5 L Cell Culture Bag	340 ml	500 ml	3.4 ml	1%
D9	NK Expansion Medium	0.5-1	2.5 L Cell Culture Bag	700 ml	1200 ml	0 ml	0%
D11	NK Expansion Medium	0.5-1	2.5 L Cell Culture Bag	800 ml	2000 ml	0 ml	0%
D14-D16	Harvested Cell（2000 ml）						

Note

- Equilibrate the medium to room temperature before use. Do not freeze-thaw cytokines more than 3 times.
- For the first 5 days, minimize cell counting, observation, and pipetting to avoid disturbing the initial growth phase of the cells.
- Autologous plasma may be substituted with hAB serum (human AB serum) or plasma replacement products. If autologous plasma is insufficient for the entire experimental process, ensure its use for at least the first 7 days.
- Due to inter-sample variability, differences in cell proliferation and purity may occur. If the culture medium turns yellow due to excessive cell growth, slightly increase the medium volume.
- When transferring cells to a new flask or bag, residual cells in the original flask can be further cultured by adding fresh medium (without plasma). After 2-3 days, suspended cells or cell clusters can be collected and transferred to the main culture system. Discard any remaining adherent cells that do not detach after 2-3 days.
- All cell culture flasks used are TC-treated.
- For optimal gas exchange and reduced metabolic waste accumulation, use larger-volume culture bag (e.g., 2 L bag for 1 L culture system; 2.5 L bag for 2 L culture system).

For research use only, not for clinical diagnosis.

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