Handling of NK Cells using NK Cell Culture Medium

Natural Killer (NK) Cell Culture Medium

The NK Culture Medium is designed for the optimal culture and expansion of primary NK cells. NK Cell Culture Medium consists of Basal Medium (90mL) plus Supplement A, B, C and D. The supplements A, B, C and D contain human serum and NK cell specific growth factors. In order to obtain complete NK Cell Culture Medium, ALL supplements need to be added to the basal medium entirely. The addition of human serum may cause the medium to appear opaque.

Storage: Store the basal medium protected from light at 2 − 8 °C. Store all supplements immediately after arrival at -20 °C. Do not freeze the basal medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the supplements to the basal medium, the shelf life of the NK Cell Culture Medium protected from light is 6 weeks at 2 − 8 °C.

NK Cell Thawing Medium

Use RPMI medium supplemented with 10% FBS to thaw NK cells. NK Cell Thawing Medium is not provided by upcyte technologies.

Application

Natural Killer (NK) cells are lymphocytes that play a critical role in the innate immune system. They are involved in viral infections, cancer, autoimmunity and adaptive immunity. Use of NK Cell Culture Medium allows the induction of NK cell proliferation with an expansion rate of up to 50 population doublings. Please note that the performance may vary as NK cell expansion is donor-dependent.

Thawing of cryopreserved NK cells

1. Pre-warm 20mL thawing medium to 37°C.
2. Additionally, pre-warm an appropriate volume (at least 5mL) of NK Cell Culture Medium for plating the cells. Leave the remaining medium in the refrigerator.
3. Carefully remove the cryovial from the storage tank. This should only take seconds.
4. Thaw cells in a 37°C water bath until all the ice has completely disappeared. Do not shake the vial or take it out of the water during thawing.
5. Spray 70% ethanol on the vial and the tube containing 20mL of thawing medium and transfer to a laminar flow-hood.
6. Transfer the thawed cell suspension (1mL) from the cryovial into 20mL NK Cell Culture Medium by gently pouring the cells into the medium.
7. Using a 1mL pipette, transfer 1mL of the NK cell medium back to the cryovial and pour the contents back into the 50mL tube. Repeat this process twice to completely remove the cells from the cryovial.
8. Pellet the cells by centrifuging at 300 × g for 10 min at RT.
9. Aspirate the supernatant without disrupting the pellet. Leave approximately 200-400µL medium on top of the cells.
10. Gently loosen and resuspend the cells without adding any extra medium by pipetting up and down the suspension 2 - 4 times. Do not vortex or shake the cells.
11. Add an appropriate volume of pre-warmed NK Cell Culture Medium to the pellet (approximately 1mL per million cells thawed) and resuspend the cells.
12. Determine cell number by e.g. using a Neubauer haemocytometer.
13. Culture NK cells in the density range of 1 x 10^5 to 5 x 10^6 NK cells/mL.
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NK cell expansion protocol

- Note: Medium should not be pre-warmed to 37 °C. Use NK Cell Culture Medium directly from the refrigerator and return to 2 - 8 °C after use.
1. Seed the cells in a standard culture plate, e.g. 1mL per well in a 24-well cell culture plate. Incubate under standard culture conditions (in a humidified incubator at 37 °C and 5% CO2).
2. Inspect the cultures daily and perform the first medium exchange after 3 - 4 days.
3. To perform a medium exchange, remove only half of the medium, taking care not to disturb the cells.
4. Replace the volume with cold NK Cell Culture Medium. Always keep unused medium at 2 - 8 °C.
5. Once the cells start to proliferate and reach a cell number of higher than 1 x 10^6 the medium might need to be changed more often.
6. The first signs of NK cell growth should be detectable after approximately 10 days.
7. To passage the cells, first break up any originated clumps by gently pipetting the cell suspension up and down and then determine the cell number. Dilute the cells to 1 x 10^6 cells per mL in NK Cell Culture Medium (NK cells can be maintained at a cell density of 5 x 10^5 - 2 x 10^6 NK cells per mL).
8. If necessary, transfer cells to a fresh culture plate of an appropriate size.
9. The expansion of NK cells typically takes 4 weeks.
10. NK cell marker expression analysis and/or functional studies can be performed during or at the end of the expansion.

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Product information

<table>
<thead>
<tr>
<th>Product</th>
<th>Supplements/Components</th>
<th>Product number</th>
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<tbody>
<tr>
<td>NK Cell Culture Medium</td>
<td>• Basal NK Cell Culture Medium (90mL)</td>
<td>MNK002</td>
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<tr>
<td></td>
<td>• Supplement A (1 mL)</td>
<td></td>
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<tr>
<td></td>
<td>• Supplement B (10 mL)</td>
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<td>• Supplement C (100 µL)</td>
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<td>• Supplement D (100 µL)</td>
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