# P3/NSI/1-Ag4-1 [NS-1] Cell Line

**Cat. No. : CL-0409**

## Origin and General Characteristics

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| **Cell Name** | P3/NSI/1-Ag4-1 [NS-1] |
| **Organism** | Mouse |
| **Sex** | Female |
| **Morphology** | Lymphoblast-like |
| **Growth Properties** | Suspension |
| **Background** | P3/NSI/1-Ag4–1 [NS-1] is a non-secreting clone of the P3X63Ag8 cell line. The cells synthesize immunoglobulin kappa light chains but do not secrete them. They are resistant to 0.1 mM 8-azaguanine and fail to grow in HAT medium. P3/NSI/1-Ag4–1 cells have been reported to be cholesterol auxotrophs owing to a deficiency of 3-ketosteroid reductase activity. |
| **Biosafety Level** | BSL-1 |

## Culture Conditions and Handling

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| **Complete Medium** | DMEM[PM150210]+10% Nutrient+1% Supplement |
| **Subcultivation Ratio** | $3\times10^5$-$5\times10^5$ cells/mL |
| **Medium Renewal** | 2 to 3 times per week |
| **Incubation Atmosphere** | Air, 95%; $CO_2$, 5% |
| **Temperature** | 37°C |
| **Freeze Medium** | Freezing Medium (Serum-free & animal origin-free)[PB180438] |
| **Storage Conditions** | For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only. |
| **Instructions** | 1. Check all containers for leakage or breakage.<br>2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation.<br>3. This cell line is a suspension cell line. Harvest the cell suspension by centrifugation; do not discard the culture medium directly. |
| **Subculturing Procedure** | Cultures can be maintained by either supplementing with fresh medium or exchanging medium via centrifugation. The recommended centrifugation conditions are 1200 rpm (approximately 250 ×g) for 3 minutes. |

## Special Features of the Cell Line

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| **Antigen Expression** | H-2d |
| **Genes Expressed** | immunoglobulin (not secreted), H-2d |

## Handling Recommendations for Cryopreserved Cells

### Preparations Before Thawing

1. Prepare the recommended complete culture medium and preheat it to 37°C for 30 minutes.
2. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.

3. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

### Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a biosafety cabinet. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

### Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in T25 flasks or 10 cm dishes.
3. Minimize the time thawed cells are kept at room temperature. Cryoprotectant must be immediately diluted or removed by centrifugation.

## Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than two weeks whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, $CO_2$ incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.

**Note:** Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.

5. After sufficient expansion, the cells should be cryopreserved as soon as possible to ensure availability of backup stocks for future experiments or in case of unexpected experimental failure. Cells in the logarithmic growth phase should be selected for cryopreservation. After cryopreservation, it is recommended to resuscitate one vial to verify post-thaw viability and confirm successful cryopreservation.

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