**Description**

Organism: *Homo sapiens*, human  
Tissue: peripheral blood  
Disease: acute lymphoblastic leukemia (ALL)  
Age: 19 years  
Gender: male  
Morphology: lymphocyte-like  
Growth Properties: suspension  

**DNA Profile:**  
Amelogenin: X,Y  
CSF1PO: 11,12  
D13S317: 9,11  
D16S539: 10,11  
D7S820: 8,11  
THO1: 8,9  
TPOX: 8,10  
vWA: 15,16  

**Batch-Specific Information**

Refer to the Certificate of Analysis for batch-specific test results.

**SAFETY PRECAUTION**

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

**Unpacking & Storage Instructions**

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

**Handling Procedure for Frozen Cells**

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

**Handling Procedure for Flask Cultures**

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt, visually examine the culture for macroscopic evidence of any microbial contamination.
Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination.

2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.

3. From this cell suspension, remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2.5 x 10^5 viable cells/mL in the shipping medium.

4. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

### Subculturing Procedure

Cultures can be maintained by addition or replacement of fresh medium. Start cultures between 2 x 10^5 cells/mL and 4 x 10^5 cells/mL maintain between 3 x 10^5 and 3 x 10^6 cells/mL. Do not allow the cell concentration to exceed 2 x 10^6 cells/mL.

### Medium Renewal

Add fresh medium every 2 to 3 days (depending on cell density).

### Cryopreservation Medium

50% culture medium + 40% FBS + 10% DMSO

### Comments

CRL-3274, N6/ADR cells, are an Adriamycin (doxorubicin)-selected, P-gp-positive (P-glycoprotein 1) variant of the NALM6 cell line. Logarithmically growing NALM6 cells were intermittently exposed to increasing concentrations of Adriamycin over a period of 15 months to generate the Adriamycin resistant cell line N6/ADR. NALM6 cells are a human B cell precursor leukemia cell line established in 1976 from the peripheral blood of a 19-year-old man with acute lymphoblastic leukemia (ALL) in relapse. N6/ADR cells are 4.6 fold resistant to Adriamycin as compared to its parental NALM6 cell line. N6/ADR cells are cross-resistant to etoposide, vincristine, Actinomycin D and mAMSA (4'-(9'­acridinylamino)methanesulfon­m­anisidide).

These cells exhibit the "classical" form of multidrug-resistance (MDR) associated with overexpression of P-gp, a membrane glycoprotein (P-glycoprotein 1) which functions as an energy-driven pump to expel anticancer agents from the cell. The lytic sensitivity and level of drug resistance of N6/ADR cells is similar to that observed for primary P-gp-positive leukemias, making these cells a good model to evaluate binding, activation and lytic events in the NK cytolytic pathway. These cells are used to investigate the function of CD24 and studying the antigen expression patterns of drug resistant leukemia cells necessary for successful bone marrow purging prior to autologous bone marrow transplantation.

### References

References and other information relating to this product are available online at www.atcc.org.

### Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

### ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is RPMI-1640 Medium (ATCC® 30-2001™). To make complete medium add the following to RPMI-1640:

- 10mM HEPES
- 70 nM Doxorubicin
- 10% Fetal Bovine Serum (ATCC® 30-2020™)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: N6/ADR (ATCC® CRL-3274™)