



# N6/ADR

CRL-3274™

Product Sheet

## Description

**Organism:** *Homo sapiens*, human

**Tissue:** Peripheral blood

**Age:** 19 years

**Gender:** Male

**Morphology:** lymphocyte-like

**Growth properties:** Suspension

**Disease:** Acute lymphoblastic leukemia ALL

**Volume:** 1.0 mL

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## Intended Use

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

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## BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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## Handling Procedures

### Unpacking and 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.

**Complete medium:** The base medium for this cell line is RPMI-1640 Medium (ATCC®)

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30-2001™). To make complete medium add the following to RPMI-1640:

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

**Note:** Doxorubicin is highly unstable in aqueous solutions at physiologic pH and temperature. Therefore, it needs to be added fresh for each use of the complete growth medium. For each day's use, add fresh doxorubicin to a final concentration of 70nM into a suitable volume of the medium, discard any unused complete growth medium.

### Example media preparation:

- Combine 500 mL RPMI (ATCC 30-2001), 56 mL FBS – not heat inactivated (ATCC 30-2020), and 6 mL 1M Gibco HEPES (ThermoFisher Scientific 15630-080 or equivalent)
- To an aliquot of the media prepared above \_ 70 uM Doxorubicin: Use 1 ul/1mL of complete culture media

### To prepare 70 uM DoxorubicinL combine the following:

- 100 ul Doxorubicin Hydrochloride (2 mg/mL, Sigma #D520)
- 5 mL Molecular Grade Water (ATCC 60-24500)

Store in working aliquots at -80C. Stable for 6 months when prepared and stored as directed. Do not repeat freeze/thaw. Do not store remaining solution after use.

**Note:** Doxorubicin is stable for only for 5 days in culture

**Note:** Doxorubicin is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised when handling it. The use of gloves and appropriate eye/face protection is recommended.

**Handling Procedure:** 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 150 to 400 x *g*; 8 to 12 minutes (200 x *g* for 10 minutes).
4. Resuspend cell pellet with the recommended complete growth medium (see the lot information on Certificate of Analysis (COA) for the culture recommended dilution ratio) and dispense into a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask as recommended on the COA. The recommended seeding density for CRL-3274 is 2.0 x 10<sup>5</sup> to 4.0 x 10<sup>5</sup> viable cells/mL. 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% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

**Subculturing procedure:**

**Subcultivation Method:** Addition of culture medium

**Subculture Restrictions:** Full fluid change must be performed at least every 5 days.

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

**Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density).

**Reagents for cryopreservation:** 50% culture medium + 40% FBS + 10% DMSO (ATCC 4-X)

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**Material Citation**

If use of this material results in a scientific publication, please cite the material in the

following manner: N6/ADR (ATCC CRL-3274)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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## Contact Information

## N6/ADR

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