



3A9

CRL-3293™

Description

3A9 is a T cell hybridoma line that was isolated in 1982 from a mouse. It is reactive against HEL [small protein hen egg-white lysozyme (HEL) composed of 129 amino acid] presented by macrophages in the context of I-A polypeptides. This cell line secretes IL-2 upon interaction with I A-bearing peritoneal macrophages that had taken up native HEL. This cell line was deposited by P Allen.

Organism: *Mus musculus*, mouse

Cell Type: hybridoma: T cell

Morphology: lymphoblast-like

Growth properties: Suspension, single cells

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

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.

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

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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.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Atmosphere: 95% Air, 5% CO₂

Seeding density: 0.5 x 10⁵ to 1.5 x 10⁵ viable cells/mL

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.

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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 ATCC-formulated DMEM (ATCC[®] 30-2002). To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC[®] 30-2020) to a final concentration of 5% FBS and 0.05 mM 2-mercaptoethanol

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 $125 \times g$ 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 cm^2 or a 75 cm^2 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% CO_2 in air atmosphere is recommended if using the medium described on this product.

Subculturing procedure: Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2×10^5 to 4×10^5 viable cells/mL. Do not allow the cell concentration to exceed 2×10^6 cells/mL.

Medium Renewal: Add fresh medium every 3 to 4 days (depending on cell density).

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: 3A9 (ATCC CRL-3293)

References

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

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