



# BRL 3A

CRL-1442™

## Description

BRL 3A is a fibroblast-like cell isolated from the liver of a rat. This cell line was deposited by SP Nissley and MM Rechler.

**Organism:** *Rattus norvegicus*, rat

**Cell Type:** fibroblast

**Tissue:** Liver

**Growth properties:** Adherent

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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 ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth

medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10% This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere. ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500 mL) and ATCC® Catalog No. 30-2021 (100 mL).

**Handling Procedure: Note:** For 24 hrs after initiating culture from a thawed ampule or after subculturing, grow the cells in culture medium with 10% fetal bovine serum. If desired, after 24 hrs, the cells may be transferred to serum-free medium.

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. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium with 10% FBS added. After 24 hrs, the cells may be transferred to serum-free recommended growth medium.
4. Transfer the vial contents to an appropriate size vessel. 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 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:** Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to

remove all traces of serum that contains trypsin inhibitor.

3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium with 10% FBS added. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 37°C. 24 hours later perform a fluid change with serum free growth medium if desired (Nissley SP, et al., 1977).

**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended

**Medium Renewal:** 2 times per week

**Note:** For 24 hrs after initiating culture from a thawed ampule or after subculturing, grow the cells in culture medium with 10% fetal bovine serum. If desired, after 24 hrs, the cells may be transferred to serum-free medium.

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a Manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

**Reagents for cryopreservation:** Complete growth medium supplemented with 10% (v/v) FBS and 5% (v/v) 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: BRL 3A (ATCC CRL-1442)

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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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## **BRL 3A**

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Product Sheet

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