



## Product Sheet

# R3 [33-10ras3] (ATCC® CRL-2764™)

### Please read this FIRST



Storage Temp.  
**liquid nitrogen**  
vapor phase

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Biosafety Level  
**2**

### 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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

### Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: R3 [33-10ras3] (ATCC® CRL-2764™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

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### Description

**Organism:** *Rattus norvegicus*, rat  
**Cell Type:** neuronal Schwann cell; immortalized with SV40 large T antigen  
**Morphology:** neuronal  
**Growth Properties:** adherent

### 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

#### 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.

**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.

1. Thaw the vial by gentle agitation in a **33°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 75 cm<sup>2</sup> tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution ratio). 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).
4. Incubate the culture at **33°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.

If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

### Handling Procedure for Flask Cultures

#### 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. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The



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shipping medium can be saved for reuse. Incubate the cells at 33°C in a 5% CO<sub>2</sub> in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 33°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.



### Subculturing Procedure

#### Protocol:

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 33°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 33°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended

**Medium Renewal:** Every 2 to 3 days



### Cryopreservation Medium

#### Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.  
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.



### Comments

#### AntigenExp:

SV40-tsA58 large T antigen [PubMed:3049071] Oncogene:

v-Ha-ras[PubMed:3049071] Products:

Myelin-associated glycoprotein (Mag); Protein and mRNA, negative [PubMed: 12210843] Myelin basic protein (Mbp); Protein and mRNA, negative [PubMed: 12210843] Myelin protein zero (Mpz) (Charcot-Marie-Tooth neuropathy 1B); Protein and mRNA, negative [PubMed: 12210843] peripheral myelin protein 22 (Pmp22); protein, negative; mRNA, positive; [PubMed: 12210843] S100 calcium-binding protein, beta (neural) (S100b) protein, positive [PubMed: 12210843]Receptors:

Nerve growth factor receptor (Ngfr) Protein and mRNA, negative

#### Comments:

Primary cells were infected with the recombinant retrovirus LJ-tsSVLT containing the temperature sensitive tsA58 large SV40 T oncogene and a gene encoding G418 resistance. Clones were selected in the presence of 0.4 mg/ml G418 and super-infected with Zipras 6 containing v-Ha-ras at the permissive temperature of 33C [PubMed:3049071]. The cells proliferate at both the permissive temperature of 33C and the restrictive temperature of 39.5C. However, the growth rate is increased and growth factor requirements are reduced at the permissive temperature [PubMed: 3049071]. At the restrictive temperature, expression of large T antigen was not detected [PubMed: 3049071]. Expression of p21-v-Ha-ras was not detected [PubMed: 3049071]. A culture submitted to the ATCC in December of 2002 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with BM Cycline. The cells were assayed for mycoplasma, by the Hoechst stain, PCR and the standard culture test, after a six-week period following treatment. All tests were negative.



### References

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



### Biosafety Level: 2

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



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### Disclaimers

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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