ATCC

Product Sheet

# RN33B (ATCC® CRL-2825™)

**Please read this FIRST**

Storage Temp.
**liquid nitrogen vapor phase**

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 DMEM:F12 Medium Catalog No. 30-2006. 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: RN33B (ATCC® CRL-2825™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

*Or contact your local distributor*

## Description

**Organism:** *Rattus norvegicus*, rat
**Strain:** Sprague-Dawley
**Tissue:** brain; medullary raphe nucleus
**Cell Type:** neuronalSV40 large T antigen transfected
**Age:** 12.5 days gestation embryo
**Morphology:** spindle-shaped at 33C; neurite-like at 37C
**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 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 $cm^2$ 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 **33°C** in a suitable incubator. A 5% $CO_2$ in air atmosphere is recommended if using the medium described on this product.

## 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 33C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

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5. Add appropriate aliquots of the cell suspension to new culture vessels.
   An inoculum of 5 X 10(3) to 8 X 10(3) viable cells/sq. cm is recommended.
6. Incubate cultures at 33C.

**Interval:** Maintain cultures at a cell concentration between 3 X 10(4) and 1 X 10(5) cells/sq. cm
**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 is recommended
**Medium Renewal:** Two to three times weekly

## Cryopreservation Medium

**Cryoprotectant Medium**

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

## Comments

RN33B is an neuronal cell line derived from medullary raphe cells by infection with a retrovirus encoding the temperature-sensitive mutant of SV40 large T antigen. The cells were cloned by serial dilution. At the permissive temperature of 33C, RN33B cells divide and express SV40 T antigen, vimentin, nestin, diffuse neuron-specific enolase, and low and medium molecular weight neurofilament immunoreactivities. At the non-permissive temperature of 37C to 39C SV40 T antigen expression is markedly decreased and RN33B cells cease mitotic activity and differentiate with phase bright cell bodies and 'neuritic-like' processes. Differentiated RN33B cells express enhanced neuronal-specific protein expression but do not synthesize astrocytic or oligodendrocytic-specific proteins. Moreover, differentiated RN33B cells returned to 33C re-express T antigen, but do not de-differentiate or begin dividing. Immunohistochemical and Northern blot analysis revealed high levels of low affinity NGF receptor protein and mRNA in differentiated RN33B cells. PCR analysis demonstrated the presence of trkB, but not trkA or trkC, mRNA in both undifferentiated and differentiated RN33B cells. The cells can be used as a model of neuronal differentiation in vitro.

## References

References and other information relating to this product are available online at 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

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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