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 33°C; neurite-like at 37°C
Growth Properties: adherent

### 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™)

### Handling Procedure for Frozen Cells

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 cm² or a 75 cm² 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% CO₂ 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 33°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
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.

Incubate cultures at 33°C from the U.S.

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

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RN33B cells express enhanced neuronal-specific protein expression but do not synthesize astrocytic or oligodendrocytic-specific proteins. Moreover, differentiated RN33B cells returned to 33°C 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 and other information relating to this product are available online at www.atcc.org.

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.

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