NB324K

CRL-3726™

Description

NB324K are SV40 immortalized cells isolated from normal neonatal kidney. NB324K cells may be used as a permissive and productive host cell line for a wide range of rodent protoparvoviruses.

Organism: *Homo sapiens*, human
Tissue: kidney
Age: neonate
Morphology: Epithelial-like and/or fibroblast-like
Growth properties: Adherent
Disease: Normal

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 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% CO_2

Handling Procedures

Complete medium:

The base medium for this cell line is ATCC-formulated DMEM Medium (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium:

- Fetal bovine serum to a final concentration of 5%
- L-Glutamine to a final concentration of 2 mM

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 150 to 400 x g for 8 to 12 minutes.
4. Carefully aspirate the supernatant and discard, leaving the cell pellet.
5. Gently resuspend the cell pellet with the appropriate amount of complete growth medium and transfer cell suspension into a vented T-75.
6. Place the flask in a 37°C incubator with 5% CO_2.

Subculturing procedure: Volumes used in this protocol are for 75 cm^2 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 PBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of 0.25% Trypsin/0.53 mM EDTA (ATCC 30-2101) to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 minutes).

   Note: This cell line is resilient to manipulation. Cells may be clumpy upon trypsinization. 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 aspirate cells by gently pipetting.
5. Centrifuge the cell suspension at approximately 150 to 400 x *g* for 8 to 12 minutes to remove dissociation agent.
6. Resuspend the cell pellet in an appropriate amount of complete culture

medium.

7. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 1.0 x 10^4 and 2.0 x 10^4 viable cells/cm^2.
8. Incubate cultures at 37°C.

Interval: Seed cells at subculture at a cell concentration between 8.0 X 10^3 and 1.5 X 10^4 cell/cm^2

Subcultivation Ratio: A subcultivation ratio of 1:10 to 1:20 is recommended

Medium Renewal: Every 2 to 3 days

Reagents for cryopreservation: Complete culture medium + 5% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: NB324K (ATCC CRL-3726)

References

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

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