If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere is recommended if the cell line is sensitive to CO₂. If the cell line is less sensitive, the incubator can be maintained at 37°C with a final concentration of 10%.

HOMO SAPIENS, HUMAN,
Tissue: embryonic kidney
Cell Type: transformed with adenovirus 5 DNA
Age: fetus
Morphology: epithelial-like
Growth Properties: adherent

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. Note: After thawing, these cells take 4 to 5 days to begin attaching to the flask. Perform first medium replacement at that time. The cells will grow in chains or small clumps that will eventually attach and spread.

Attachment can be enhanced by using Corning CellBIND flasks.

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 growth medium and spin at 350 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete growth 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 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

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. The cell line does not adhere to the substrate when left at room temperature for any length of time. Therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37°C.

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 shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.

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%.

To make the complete growth medium, add the following:

- 250 mL fetal bovine serum
- 9.0 mL complete growth medium
- 0.25 mL 10× ATCC-supplied complete growth media supplement

The cell line does not adhere to the substrate when left at room temperature for any length of time. Therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37°C.

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3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

These cells adhere poorly to standard tissue culture flasks. Attachment can be enhanced by using Corning CellBIND® flasks.

Volumes used in this protocol are for 75 cm² flasks; 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 Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 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. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

**Subcultivation ratio:** A subcultivation ratio of 1:5 to 1:20 is recommended.

**Medium renewal:** every 2 to 3 days

**Cryopreservation 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**

The HEK293S GntI- cell line was established by methanesulfonate mutagenesis followed by Ricin selection. HEK293S GntI- cells do not have N-acetyl-glucosaminyltransferase I (GnTI) activity and therefore lack complex N-glycans. This cell line has been successfully used to overexpress a wide variety of mammalian membrane proteins. There were two versions of HEK293S GntI- cells in the original publication. One was HEK293S GntI- (ATCC CRL-3022), and the other version was HEK293S GntI- variant constructed with tetracycline-inducible system. CRL-3022 does not contain tetracycline-inducible system or pcDNA6-TR vector.

**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.

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