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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.

**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.
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. Recommended use of Corning® T-75 flasks (catalog #430641). Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes are given for a 75 cm² flask. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

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 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. An inoculum of 1 x 10⁴ to 4 x 10⁴ viable cells/cm² is recommended.
6. Incubate cultures at 37°C. Subculture when cell concentration is between 6 and 7 x 10⁴ cells/cm².

**Subcultivation Ratio:** 1:6 to 1:10 weekly

**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

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present.

The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit.

The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment fromnts 1 to 4344 is integrated into chromosome 19 (19q13.2).

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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