Unpacking & Storage Instructions

Handling Procedure for Frozen Cells

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 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.

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. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: McA-RH7777 (ATCC® CRL-1601™)
Subculturing Procedure

Heavy monolayer sloughs off; subculture before 70% confluency. Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove culture medium with floating cells to a centrifuge tube.
2. If any cells are attached, tap flask gently or if necessary add 2.0 to 3.0 mL of 0.25% Trypsin-0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed.
3. Add 2.0 to 3.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. To remove trypsin-EDTA solution, transfer cell suspension to the centrifuge tube with the medium and cells from step #1 and spin at approximately 125 x g for 5 to 10 minutes.
5. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
6. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 weekly is recommended

Medium Renewal: Add medium every 2 to 3 days, do not discard floating cells.

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

Addition of glucocorticoids (dexamethasone) to the medium accelerates cell proliferation and reduces alpha fetoprotein production.

References

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

Biosafety Level: 1

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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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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