Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by
   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.
3. Transfer the vial contents to a 75 cm² tissue culture flask and dilute with the recommended complete
   culture medium (see the specific batch information for the recommended dilution ratio). 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 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).
4. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended
   using the medium described on this product sheet.
5. If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell
   suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes.
   Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio
   recommended in the specific batch information.

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).
Subculturing Procedure

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 and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which 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 to remove all traces of Trypsin-EDTA. If necessary, use a pipette to detach cells, but do not agitate the cells. Resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
5. Place culture vessels in incubators at 37°C. Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended

Medium Renewal Ratio: 2 to 3 times per week

Comments

This cell line is a suitable host for transfection, especially by SV40 vectors.

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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Please read this FIRST

Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. 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: CV-1 (ATCC® CCL-70™)