YUMM4.1 (ATCC® CRL-3366™)

Please read this FIRST

Storage Temp. liquid nitrogen vapor phase

Biosafety Level 1

Description

Organism:  *Mus musculus*, mouse
Strain: c57bl/6j
Tissue: skin
Disease: melanoma
Gender: female
Morphology: epithelial-like
Growth Properties: adherent

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 DMEM: F12 (ATCC 30-2006). To make the complete medium add the following components to the base medium:
- 56 mL fetal bovine serum (FBS) (ATCC 30-2020)
- 5.6 mL NEAA (Gibco Cat# 11440-076)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: YUMM4.1 (ATCC® CRL-3366™)

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

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 Frozen Cells

To ensure 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.

Unpacking: If the frozen cells arrive in a dry ice packaging, proceed to the thawing and the medium preparation.

Storage: To store the frozen cells, the packaging containing the vial should be placed in a box containing liquid nitrogen. Ensure that there is enough liquid nitrogen (at least 100 mL) in the box to cover the vial. Place the box in a liquid nitrogen storage freezer (at least -130°C).

Thawing Procedure

1. Remove the frozen cells from the talent nitrogen vapor phase and place the vial in a 37°C water bath. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from liquid nitrogen and place the vial in a warm (37°C) water bath. Thawing should be rapid (approximately 2 minutes).
3. Transfer the vial to a centrifuge tube containing 9.0 mL complete culture medium, and spin at approximately 150-400 x g for 8 to 12 minutes.
4. Resuspend the cell pellet with the recommended complete medium (see the specific batch information for the culture 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 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). 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.

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. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

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. Cultures can be established between 2 x 10⁴ and 4 x 10⁴ viable cells/cm².
6. Incubate cultures at 37°C.
Interval: Maintain cultures at a cell concentration between $8 \times 10^3$ and $6.0 \times 10^5$ cell/cm$^2$.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

Note: Subculture at confluence ≤90% as cells tend to lift off in sheets at higher confluence

Culture media + 5% DMSO (ATCC 4-X)

This cell line is genetically modified.

Tyrosinase:CreERT2 transgene restricts recombination to melanocytes.

Pten$^{+/\text{m}}$ (conditionally inactivated)

Cdkn2a$^{+/\text{m}}$ (conditionally inactivated)

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

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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