Product description

Cells derived from C57 Bl6 mice carrying a stable insertion of the conditional immortalising gene H-2Kb-tsA58, which describes a temperature-sensitive variant of the SV40 immortalising gene that encodes the large tumour antigen under the control of the gamma interferon-sensitive MHC Class 1 promoter. The transgenic mouse is called the Immortomouse™ (Jat et al 1991 Proc. Nat. Acad. Sci. USA 88, 5096-5100)

Name: UB-UE1 Cell Line

Organism: Mouse

Tissue: Vestibular epithelium (utricular macula)

Disease: Hearing loss

Growth properties: Adherent

Production details: Derived from vestibular epithelium (utricular macula) using thermolysin treatment and dissection to ensure origin from sensory epithelial supporting cells at post-natal day P2. At this stage the supporting cells are normally post-mitotic but retain the ability to differentiate as sensory hair cells and or supporting cells. UB/UE-1 was characterised by timed expression under differentiating conditions in vitro of a combination of gene and protein markers for epithelial cells and for inner ear sensory cells. These include Cytokeratin, Vimentin, Myosin VIIa, Myosin VI, Brn3c, α9AChR and a combination of functional ion channels (see references). Screened with Affymetrix mouse Micro-arrays. The cells differentiate neonatal stage hair cell and supporting cell phenotypes under differentiating conditions in vitro.

Model: Immortalized cell line

Conditional: Yes

Application: Inner ear development; Gene expression and function of inner ear-specific genes; In vitro screening for gene activation and promoter analysis; Ototoxicity studies (prescribed drugs and agents that ameliorate their affects)

Biosafety level: 1

Cellosaurus id: CVCL_D807

Contributor(s)

Inventor: Matthew C Holley
Institute: University of Bristol; University of Sheffield

Properties

Product format: Frozen

Unpacking and storage:

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.

Recommended medium: MEM with 10% FCS, 50 U/mL IFN-y, L-glutamine.

Subculture: Remove the medium from the cells. Rinse the flask twice with Hanks Balanced Salt Solution to remove any trypsin inhibitors present in the FCS. Decant off the Hanks and remove the last drops with a pipette. Add trypsin and leave the flask at room temperature or in the incubator as required. (The flask may be shaken vigorously at periodic intervals to accelerate the removal of the cells from the surface of the flask. The progress of cell detachment can be followed by observation under microscope). When the cells are detached, add excess of serum-supplemented growth medium (MEM+10% FCS). (Trypsin inhibitors in the serum will prevent excessive damage to the cells by trypsin). Centrifuge at 1000g for 3min. Remove the supernatant and re-suspend the cells in fresh medium (2-5 mL for 25 cm^2 or 5- 10 mL for 75 cm^2 vented cap flasks). Let cells grow until 80-90% confluent (between 3 days to a week).

Culture conditions: 33.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2 for maintenance as undifferentiated cells. To culture cells under differentiating conditions, replate trypsinized cells in MEM/FCS without IFN-γ at 39°C.

Cryopreservation medium: 10% DMSO in FCS.

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension dropwise to a 15 mL conical tube containing 9 mL pre-warmed MEM using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 15 mL tube.
7. Centrifuge the cell suspension at **1000 x g for 3 minutes** at room temperature.
8. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Re-suspend the cell pellet into 2ml of MEM: 10%FCS: 100 U/ml IFN-γ.
9. Transfer into a 25 cm^2 vented cap flask. Incubate at 33°C for 2-3 days, until cells are about 80% confluent. (If cells are harvested at confluence, there may be some interruption of cell growth, which may result in a lag phase on subculture).
10. To maintain cultures, if cells are seeded at 1-2 x 10^5 in a 75 cm^2 flask, they may need to be passaged about once a week.
11. To differentiate cells, sub-culture them at desired density in culture medium without IFN and place at 33°C. When cells are established, transfer to 39°C. It takes about 2-3 days at 39°C for complete inactivation of the T antigen.

References

- Rivolta and Holley. 2002. J Neurobiol. 53:306-18. PMID: 12382283
- Hackett et al. 2002. Exp Cell Res. 278(1):19-30. PMID: 12126954
- Lawlor et al. 1999. J Neurosci. 19(21):9445-58. PMID: 10531448

Material Citation

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