Product description

US/VOT-N33 Cell Line is a conditionally immortalized cell line representing migrating neuroblasts derived from the ventral otocyst of the Immortomouse™ at embryonic day 10.5 (E10.5). At this developmental stage, the sensory epithelia of the inner ear have not yet differentiated, and the ventral otocyst epithelium is competent to give rise to auditory neurons and epithelial cells. The cell line was established to model early inner ear development and mechanisms underlying hearing loss in mammals.

The cells carry a stable insertion of the temperature-sensitive SV40 large T antigen (H-2Kb-tsA58) under the control of a γ-interferon-sensitive MHC Class I promoter, allowing conditional immortalization. The growth temperature and presence or absence of γ-interferon are critical for phenotype:

Proliferating conditions: 33°C with γ-interferon

Differentiating conditions: 39°C without γ-interferon

The cell line has been extensively characterized using Affymetrix mouse microarrays and has demonstrated functional integration following transplantation to the cochlear nerve in vivo.

Name: US/VOT-N33 Cell Line

Organism: Mouse

Tissue: Embryonic

Disease: Hearing loss/ deafness

Growth properties: Adherent

Production details: Homozygous male Immortomouse™ (originally derived from injected oocytes of CBA/Ca × C57BL/10 mice) were time-mated with wild-type C57BL/6 female mice to produce heterozygous offspring. Embryos were harvested at E10.5, and ventral otocysts were dissected under sterile conditions.

Explants were cultured in medium containing 50 U/mL γ-interferon at 33°C to support expression of the conditional immortalizing gene (H-2Kb-tsA58). Selection of explants was based on expression of tissue-specific markers, including the transcription factor GATA3, 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); function of inherited deafness mutations; functional analysis of ion channels, receptors and signalling pathways in vitro.

Biosafety level: 1

Cellosaurus id: CVCL_VC37

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Contributor(s)

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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 and move to 39°C when cells are established. It takes 2-3 days at 39°C for complete inactivation of T antigen.

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

- Sekiya et al. 2007. Eur J Neurosci. 25(8):2307-18. PMID: 17445229
- Nicholl et al. 2005. J Neurosci 22:343-353. PMID: 16045487
- Lawoko-Kerali et al. 2004. Dev Dyn. 231(4):801-14. PMID: 15499550

Material Citation

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