Product description

Immortalized human melanocyte cell line established from neonatal foreskin by introduction of retroviral construct carrying a geneticin resistance gene and containing HPV16E6E7 open reading frames.

Name: Immortalized Human Melanocyte [PIG1] Cell Line

Organism: Human

Gender: Male

Tissue: Neonatal foreskin

Disease: Skin Cancer

Growth properties: Adherent

Production details: Melanocyte cultures were established from neonatal foreskin using standard methods. Melanocytes from passage 12 were transfected with HPV16 genes E6 and E7 using the retroviral construct LXCN16E6E7. The E6E7 genes are under the control of the MMLV promoter-enhancer sequence. In addition, the vector contained a geneticin resistance gene. The retroviral particles were produced by the packaging cell line PA317. The critical concentration of geneticin for transformed selection was 1mg/ml.

Model: Immortalized cell line

Biosafety level: 1

Cellosaurus id: CVCL_S410

Contributor(s)

Inventor: Pranab K Das
Institute: Amsterdam University

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: Ham's F10 medium supplemented with 10ng/mL tetradecanoyl phorbol 13-acetate (TPA), 0.1mM 3-isobutyl-methyl-xanthine (IBMX), 1% vol/vol Ultroser G, 2mM glutamine, 100 IU/mL penicillin and 100 ug/mL streptomycin.

Subculture: Split confluent cultures 1:4 using Trypsin-EDTA solution; 5% CO2; 37°C. As these cells are cultured in serum-free media, use a trypsin inhibitor e.g. soyabean trypsin inhibitor to inactivate the trypsin.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Cryopreservation: The cells provided by CancerTools.org are frozen in Recovery™ Cell Culture Freezing Medium (Gibco™, 12648010), at a density of 1 x 10^6 cells/mL. The cell line can also be cryopreserved in serum containing 10% DMSO.

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 to a 50 mL conical tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
6. Wash by adding 15 - 20 mL of medium **dropwise**, while gently swirling the tube.
7. Centrifuge the cell suspension at **250 x g for 5 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. Resuspend the cell pellet by gently flicking the tube.
9. Gently add required volume of culture medium and transfer to a suitable cell culture flask.

References

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Material Citation

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