Product description

The melan-a cell line is an immortal melanocyte cell line derived from embryonic mouse skin. It was the first established non-tumorigenic mouse melanocyte cell line and has become a widely used model for melanocytes studies. Melanocytes are pigment-producing cells located in the bottom layer of the skin's epidermis and in other tissues. The melan-a cells display the pigmentation and morphology typical of normal melanocytes, expressing both gp100 and the melanocyte marker melan-a. They are diploid in chromosome number and syngeneic with C57BL mice and B16 melanoma sublines, facilitating comparative investigations. These cells are visibly pigmented, smaller than B16 melanoma cells and retain all tested characteristics of normal melanocytes, except senescence. Among retained features, they also display a proliferative response to cholera toxin in the presence of tetradecanoyl phorbol acetate (TPA). The availability of robust cellular models that reflect normal skin melanocytes, such as the melan-a cell line, is essential for studying the etiology and abnormalities of skin cancers and melanomas, allowing comparisons at a molecular and cellular level.

Name: melan-a cell line

Cancer: Skin cancer

Cancers detailed: Melanoma

Organism: Mouse

Tissue: Embryonic skin

Growth properties: Adherent

Model: Immortalised line; Non-tumorigenic in syngeneic and nude mice

CRISPR Edited: No

Biosafety level: 1

Cellosaurus id: CVCL_4624

Contributor(s)

Inventor: Dorothy Bennett
Institute: St George's University of London

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 vapour, until ready for use.

Recommended medium: RPMI 1640 gassed with 10% CO_2 (pH 6.9-7.0), 2 mM glutamine, 10% FCS (Important: do not heat the serum, nor use heat-inactivated serum, as this destroys its stimulatory activity for melanocytes),

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200 nM TPA. *Also requires tyrosinase inhibitor phenylthiourea (PTU) for freeze and thaw.*

Low pH is essential for pigmented melanocytes. If pH of stored medium rises noticeably (red or pink colour), re-gas from cylinder of 10% CO_2 before use.

Notes:

- Use of the **tyrosinase inhibitor phenylthiourea (PTU)**, sold by Sigma as phenylthiocarbamide. Cells are grown with PTU (100-300 µM depending on level of pigmentation) for a few days, ideally a whole passage, before freezing, and after thawing for a few hours or days. It is also recommended to add PTU at 200 µM to the solutions used for freezing and thawing the cells. This combination significantly increases recovery rates. The cells become depigmented with the use of PTU and pigmentation returns a few days after removing. PTU is dissolved in ethanol at 10-100 mM and stored as aliquots at -20° C, or briefly at 4° C (The 100 mM solution precipitates when chilled and should be warmed before use).

- Do not use DMSO as a solvent for medium supplements as DMSO is highly toxic to melanocytes. TPA can be dissolved in ethanol.

- Optional: Adding extra phenol red (water-soluble form, add 7.5 µg/mL from 3 mg/mL stock) to the RPMI medium helps to monitor pH.

Subculture: Passage at ~80-85% confluency (doubling time ~36-48 hrs). Carefully remove the medium and rinse the flask or dish with 0.5 vol. of 1X PBS without $CaCl_2$ and $MgCl_2$ (PBSA), where 1 volume is the normal volume of medium for the vessel being sub-cultured, e.g. add 7.5 mL PBSA for a 15-mL flask. Aspirate after the rinse. Gently add 0.5 vol of 250 µg/mL trypsin and 200 µg/mL EDTA in PBSA. Quickly remove most of this, replacing enough to keep the surface wet (~0.2 vol.), and incubate in a 37°C incubator for 3-5 minutes.

Inspect the culture by microscope and check for complete detachment of cells by gently rocking the culture. Do not tap or bang, but incubate further if needed, until detached (or maximum 10 min.) Add 0.5 vol. of cold growth medium (with 10% FCS, neutralizes trypsin) to the culture and wash/pipette up and down to make a single-cell suspension. Transfer to a suitable tube or round flask, e.g. 20 mL universal. Rinse culture vessel with further growth medium/FCS to make up 1 vol. total and add to the suspension. Swirl to mix evenly. Count the cell density using a haemocytometer(s), keeping suspension on ice while counting, to prevent attachment to the tube. Dilute, add mitogens and replate at ~1.5 x10^4/mL. Change medium every 4 days.

Cryopreservation: Freeze at 0.5 – 1 x10^6 cells/mL in culture medium with 7.5% DMSO and 200 µM PTU. Immediately place vials in insulated container at -70° C. Leave overnight (or up to a few days is ok without loss of viability), then transfer to liquid nitrogen freezer. Ensure pH of culture medium is not too high (even for a few minutes). Do not use partially de-gassed medium, especially for the actual freezing medium.

Culture conditions: 37.0°C ± 1.0°C incubator with 10.0% ± 1.0% CO_2

Handling instructions

1. Prepare recommended growth medium to thaw and expand the cells. The cells can grow on normal tissue culture-ware surfaces without any additional coating.
2. Remove the vial of frozen cells from liquid nitrogen and thaw rapidly by incubating in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a microbiological safety cabinet, use a 1 or 2 mL pipette to transfer the cells into a sterile 25 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

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5. Add 1 mL of growth medium **dropwise** into the conical tube with swirling over approximately 30 second. Then add a second mL also dropwise over 15 seconds, and finally 18 mL also dropwise over 30 seconds. **IMPORTANT: Do not add the entire volume of medium all at once to the cells**. This may result in decreased cell viability due to osmotic shock.
6. Centrifuge the tube at 500 x g for 5 minutes to pellet the cells. Decant as much of the supernatant as possible.
7. Resuspend the cells in fresh medium and plate out at a density of 3 x 10^4 cells/mL in a T25 flask.
8. Add 200 µM PTU to the culture medium for the first few days after thawing, until first passage and then use medium without PTU. Pigmentation should reappear.

References

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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: melan-a cell line, was invented by Dorothy Bennett (CancerTools.org #153599, Bennett et al. 1987).

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