Product description

The melan-Ink4a-Arf1, -2, -3 and -4 lines are immortal mouse melanocyte lines derived from the skin of neonatal Ink4a-Arf–/– (*Cdkn2a* null) C57BL/6J mice. Each line was derived independently from a different littermate of this background. These cells are visibly pigmented and display morphology typical of normal melanocytes. The *Ink4a-Arf (Cdkn2a)* locus encodes two key effectors of cellular senescence ($p16^{INK4A}$ and ARF) and is often mutated in a range of human cancers including melanoma. *Ink4a-Arf* null cells are naturally immortal through this deletion, yet otherwise diploid, not having spontaneously immortalized through unknown genetic changes. Therefore, these melanocytes can be used as a model of normal skin melanocytes in studies focussing on skin cancers and melanomas. They are also a useful tool for studies of pigmentation genetics and as a control for numerous other lines in the collection, as many of these have also been derived from mice that are null for *Ink4a-Arf*.

Name: Melan-Ink4a-Arf2 cell line

Organism: Mouse

Tissue: Skin

Growth properties: Adherent

Morphology: Small, oval-fusiform

Model: Immortalised non-cancerous cell lines

CRISPR Edited: No

Biosafety level: 1

Contributor(s)

Inventor: Elena Sviderskaya
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), 200 nM TPA, 200pM cholera toxin (CT). 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 also 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 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 or at least every 2 weeks. 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 subcultured, 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 ~3 $x10^4$/mL.

Cryopreservation: Freeze at 0.5 - $1x10^6$ 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 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Add 1 mL of growth medium **dropwise** into the 15 mL 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 media 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 at a density of 3 x 10^4 cells/mL per 5 mL total volume 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

- Sviderskaya EV, et al. J Natl Cancer Inst. 2002,94(6):446-54. PMID: 11904317

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Melan-Ink4a-Arf2 cell line, was invented by Elena Sviderskaya (CancerTools.org #161993).