

Product description

The GFP-KSC4 Keratinocyte Stem Cell line is a murine cell line that can be used for the generation of in vitro epidermal 'skin equivalents' to be used as screening tools to look at the activity of NCE's and/or NBE's of dermatological interest, and/or to screen for such NCE and/or NBE in high throughput systems. It may also be of use to non-pharmaceutical healthcare and/or cosmetic companies involved in the personal vitality and/or beauty product development segments.

Name: GFP-KSC4 Keratinocyte Stem Cell line

Research fields: Drug development; Stem cell biology

Organism: Mouse

Tissue: Skin

Growth properties: Adherent

Model: Transgenic

Conditional: No

CRISPR edited: No

Production details: Keratinocytes were isolated from neonatal homozygous C57BL/6-Tg(CAG-EGFP)10sb/J mouse skin. The cell line was established by growing cells for the first 8 passages in co-culture with 3T3-J2 fibroblast feeder cells. From passage 9 onwards cells were grown without feeder cells.

Cellosaurus ID: CVCL_W142

Contributor(s)

Inventor: Julia Reichelt

Institute: Newcastle 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: Low calcium medium (FAD): DMEM/Ham's F12 with 10% FCS, 0.18 mM adenine, 0.5 µg/ml hydrocortisone, 5 µg/ml insulin, 10⁻¹⁰ M cholera toxin, 10 ng/ml EGF, 2 mM glutamine and 1 mM pyruvate.

Subculture: Wash monolayer with PBS (without Ca²⁺ and Mg²⁺), incubate with EDTA solution for 5 min at room temperature and finally incubate for 8-12 min with 0.05% trypsin/0.02% EDTA at 37°C until they come off easily. Add complete FAD medium and resuspend the cells by pipetting up and down 3-5 times. After centrifugation for 5 min at 220 g resuspend the pellet in complete FAD medium and seed the cells onto collagen I-coated dishes.

Seed new cultures at $1.5\text{--}3 \times 10^4$ cells/cm².

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO₂

Handling instructions

Thawing:

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 when the **ice crystals are slightly dissolved** 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. Add the cell suspension to prewarmed culture medium in a 15 mL tube, dropwise, to minimize shock.
5. Repeat until the ice crystals have been completely dissolved.
6. Centrifuge for 5 min at 220 g and discard the supernatant. Resuspend in complete FAD medium and transfer to culture flasks.

References

- Reichelt and Haase. 2010. Methods Mol Biol 585:59-69. PMID: 19907996
- Okabe et al. 1997 FEBS Letters 407:313-9. PMID: 9175875
- Hoehner T et al. 2011. Stem Cell Rev. PMID: 21874280
- Vollmers A et al. 2011. PMID: 21892602

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: I GFP-KSC4 Keratinocyte Stem Cell line, was invented by Julia Reichelt at the Newcastle University (CancerTools #151713).

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

While CancerTools.org has made all reasonable efforts to ensure that the information provided by CancerTools.org and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.