Product description

The K38 Keratinocyte Cell Line has been established from the skin of neonatal WT mice. The prospective commercial application of the line is in 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 Murine keratinocyte stem cell line.

Name: K38 Keratinocyte cell line

Organism: Mouse

Tissue: Neonatal skin

Growth properties: Adherent

Model: Primary cell line

Production details: Keratinocytes were isolated from neonatal wild-type BALB/c 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_W141

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: The cell line is grown in Keratinocyte basal medium (low-calcium) (140116) supplemented with 10% FBS / FCS [Chelex 100 (Biorad)-treated to remove Ca^{2+} ions], 0.18 mM adenine, 0.5 µg/mL hydrocortisone, 5 µg/mL insulin,10 -10 M cholera toxin, 10 ng/mL EGF, 2 mM L-Glutamine, 1 mM pyruvate, 100 U/ml penicillin and 100 µg/ml streptomycin.

Seeding density: 3-5 x 10^4 cells/cm^2

Subculture routine: Wash monolayer with PBS (without Ca^{2+} and Mg^{2+}), incubate with EDTA solution (0.02% EDTA) for 5 min at room temperature and then incubate for 8-12 min with 0.05% trypsin/0.02% EDTA at 37°C until the cells come off easily. Add complete Keratinocyte medium and resuspend the cells by pipetting up and down 3-5 times. After centrifugation for 5 min at 220 x g, resuspend the pellet in complete Keratinocyte medium, and seed the cells at 3-5 x 10^4cells/cm^2 onto collagen I-coated plastic culture dish. Cultures are split 1:2 or 1:3,

at confluency/2-3 times per week.

Recommendations for cryopreservation: Freeze in 10% DMSO and 90% FBS/FCS (Chelex treated), at high density (5-10 x 10^6 cells/mL). To avoid thawing into large volumes of medium it is recommended to freeze 0.3-1 mL of cell suspension per cryovial.

Culture conditions: 32.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

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. Gently add thawed cells to sufficient complete medium in a suitable **collagen I-coated** cell culture flask (calculate volume so that end concentration of DMSO does not exceed 0.5%) and replace with fresh medium within 24 hours.
4. In case dilution in high volume is not possible (if the frozen cell suspension volume is greater than 1 mL, for example), thawed cells can be pipetted into 5 ml of medium in a 50 ml falcon tube and then centrifuged and the pellet resuspended in 5 ml complete medium for seeding in a T25 flask.

Important Notes:

1. Keratinocytes must not be kept for an extended time in suspension, as this can induce differentiation. Keeping the time short from thawing to seeding is therefore of key importance.
2. It is critical that these cells are cultured at 32°C.
3. Coating material other than collagen-I has not been validated for use with these cells.
4. The Ca^{2+} depletion of FBS/FCS used is essential because serum contains high calcium levels (up to 2.5 mM), which is then diluted 1:10 in the medium resulting in a final concentration of at least 0.25 mM. To avoid differentiation of the keratinocytes, the calcium level needs to be approximately 0.05 mM, which is the minimum required for the normal functioning of the cadherins (cell adhesion molecules).

References

- Vollmers et al. 2012. Stem Cell Rev. 8(2):402-13. PMID: 21892602
- Höher et al. 2012. Stem Cell Rev. 8(2):426-34. PMID: 21874280
- Reichelt et al. 2010. Methods Mol Biol. 585:59-69. PMID: 19907996

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: K38 Keratinocyte cell line, was invented by Julia Reichelt (CancerTools.org #151682).