Designation: **SK-MEL-1**

CLS order number: Cryovial: 300424
Vital: 330424

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	29 years old
Gender:	Male
Tissue:	Melanoma, malignant; skin; from metastatic site: lymphatic system
Morphology:	Spherical
Growth Properties:	Suspension
Description:	This cell line was established in 1966 by F. Oettgen and associates using cells from the thoracic duct of a patient. Pigment granules relating both to synthesis and to phagocytosis are present. According to our sequencing, WB and PCR results this cell line carries a BRAF V600E mutation. Cells are N-Ras wildtype.
Culture Conditions and Handling	
Culture Medium:	EMEM supplemented with 2 mM L-glutamine, 1% Non-Essential Amino Acids, 1mM sodium pyruvate and 10% fetal bovine serum (MG-10, CLS order number 820100).
Subculturing:	Cultures can be maintained by addition or replacement of fresh medium. Establish new cultures at 1 x 10^5 cells/ml and maintain at 2-5 x 10^5 cells/ml.
Split Ratio:	A ratio of 1:2 to 1:4 is recommended
Fluid Renewal:	2 to 3 times weekly
Freeze Medium:	CM-1 (CLS order number: 800150, 50ml)
Sterility:	Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Special Features of the Cell Line	
Tumorigenic:	Yes, in nude mice; forms pigmented malignant melanomas; also forms tumors in the cheek pouch of cortisone treated hamsters
Genotype Status:	V600E type BRAF Mutation was determined by DNA based methods (sequencing, RT-PCR) and protein based methods (Western Blot)
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
DNA Profile (STR) :	Amelogenin: X,Y CSF1PO: 12,13 D13S317: 11 D16S539: 11,12 D5S818: 12,13 D7S820: 12 THO1: 6 TPOX: 11 vWA: 16,17 D3S1358: 14,16 D21S11: 29, 32.2 D18S51: 13,16 Penta E: 7,21 Penta D: 11,13 D8S1179: 13,16 FGA: 18,20
Antigen Expression:	Blood Type A; Rh+ Antibody to this line was detected in 63% of patients with malignant melanoma and in 10% of patients with other diseases.
Isoenzymes:	PGM3, 1; PGM1, 1; ES-D, 1; AK-1, 1; GLO-1, 1-2; G6PD, B;
Genotype status:	V600E type BRAF Mutation was determined by DNA based methods (sequencing, RT-PCR) and protein based methods (Western Blot). Nras wt.
Products:	Melanin
References:	

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Oettgen HF et al. Suspension culture of a pigment-producing cell line derived from a human malignant melanoma. J Natl Cancer Inst 41: 827-43, 1968.

Recommendations for handling of suspension cells following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) may be stored in liquid nitrogen after arrival.
If immediate culturing is intended, please follow these instructions:
Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath.
From now on, all operations should be carried out under aseptic conditions.
Immediately transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium. Resuspend the cells carefully. The cells may be spun down at 250xg for 3 minutes (this depends on the cell line used). After centrifuging, aseptically remove the supernatant and add 10 ml of fresh cell culture media. Carefully resuspend the cells and distribute into one 25cm^2 cell culture flask. Incubate at 37°C/5% CO_2.
Subculture as soon as the cell concentration has reached 1 x 10^6 cells/ml. It is recommended to distribute the cells into new flasks containing fresh medium thus diminishing the amount of dead cells and cell debris. Adjust to a cell concentration of 1-2 x 10^5 cells/ml depending on the specification given for the cell line.
After about 1-2 times of sub-culturing as recommended the percentage of viable cells should be > 90%.

Proliferating Cultures

Immediately after receipt the cell concentration should be determined. If the cell concentration already has reached a value of 1 x 10^6 cells/ml or even more, subculture the cells as described above. Remove the entire content of the flask and centrifuge at 300xg for 3 minutes.
Resuspend the cell pellets as suggested under subculture procedures described on the appropriate datasheet.

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.