

MEL-CLS-3 | 300293

General information

Description	This cell line was established as in vitro cell line from the human melanoma xenograft MRI-H-221 of a primary malignant melanoma by CLS in 1998.
Organism	Human
Tissue	Skin
Disease	Amelanotic melanoma
Synonyms	MRI-H-221

Characteristics

Age	Unspecified
Gender	Male
Ethnicity	Caucasian
Growth properties	Adherent

Identifiers / Biosafety / Citation

Citation	MEL-CLS-3 (Cytion catalog number 300293)
Biosafety level	1

Expression / Mutation

Protein expression	p53(+)
Tumorigenic	Yes, in nude mice
Viruses	Negative for: Sendai, Ektromelie, Polyoma, K-Virus, Kilham, Reo 3, PVM, LCM, M.pulmonis, MVM, Theiler's GD VII, toolan's H-1, MHV, LDV, RCV/SDA, M-Adenovirus, B.piliformis

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Mutational profile V600E type BRAF Mutation was determined by DNA based methods (sequencing, RT-PCR) and protein based methods (Western Blot)

Handling

Culture Medium	DMEM
Medium supplements	10% FBS, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO ₃ , w: 1.0 mM Sodium pyruvate
Passaging solution	Accutase
Subculturing	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add TrypleExpress (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at 37 degree Celsius for 10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split ratio	A ratio of 1:2 to 1:4 is recommended
Seeding density	1 x 10 ⁴ cells/cm ²
Fluid renewal	Every 3 days
Freezing recovery	After thawing, plate the cells at 5 x 10 ⁴ cells/cm ² and allow the cells to recover from the freezing process and to adhere for at least 48 hours.
Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Handling of cryopreserved cultures The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius 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. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. 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 (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.

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Handling of proliferating cultures

One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility

Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.

STR profile

Amelogenin: x,Y
CSF1PO: 10,11
D13S317: 11,13
D16S539: 10,13
D5S818: 11,12
D7S820: 7,10
TH01: 9.3
TPOX: 8
vWA: 17,18
D3S1358: 16,18
D21S11: 28,31.2
D18S51: 16,17
Penta E: 14
Penta D: 12,13
D8S1179: 12,13
FGA: 21,25