Patient-Derived Melanoma Tumor Cell Lines

Cell Culture Protocol

The basics of cell culture for patient-derived melanoma cell lines share certain similarities, however, cell culture conditions vary typically for each melanoma cell line. Deviating from the culture conditions required for a particular melanoma cell line can result in different phenotypes being expressed. We recommend that you acquaint yourself with each cell line of interest, and carefully follow the cell line specific instructions provided with each product.

Basic Equipment

- Biosafety cabinet or laminar-flow hood
- Incubator (humidity controlled CO_2 incubator recommended)
- Water bath at 37°C
- Centrifuge
- Refrigerator and freezers (–20°C and -80°C)
- Cell counter
- Inverted microscope
- Liquid nitrogen (N_2) freezer or cryo-storage container
- Cryo-freezing chamber
- Sterilizer/autoclave

Additional Supplies

- Sterile cell culture vessels (e.g., T-25 flasks, Petri dishes, multi-well plates, conical tubes)
- Liquid nitrogen storage Dewar and cryo-vials
- Pipettes and tips
- 70% ethanol
- Syringes and needles
- Waste containers
- Media, sera, and reagents (see cell line specification sheet for appropriate media components.)

PROCEDURE

Thawing Frozen Cells

The thawing process is stressful to frozen cells, and using a good technique and working quickly should ensure that a high percentage of cells survive the procedure.

Remove vials containing viable cells from liquid nitrogen storage tank and immediately place on dry ice.

- Before starting the procedure, label and prepare a 15 mL sterile conical tube containing 10 mL of DMEM supplemented with 10% Fetal Bovine Serum (FBS; cat # FBS-01-0100) for each vial being thawed.
- To thaw a vial, place vial into a 37°C water bath until the frozen cells begin to thaw.
- Wipe the outside of the vial with 70% ethanol (alternatively isopropanol may be used) before placing it in the cell culture hood.
- Immediately transfer the cell suspension into a 15 mL conical tube containing 10 mL of DMEM/10% FBS, and then centrifuge at 1500 rpm (500 x g) for 5 minutes at room temperature.
- Discard the supernatant and resuspend cell pellet in 5 mL of Tumor Specialized Media with FBS (heat inactivated).
- Transfer the suspended cells into a T-25 flask. Place the flask in a humidified incubator (5% CO_2) at 36°C overnight.
- Check the flask after 24 hours for attachment of cells to the flask. If cells are attached, remove the media and add fresh media to the flask.

Culturing Adherent Cells

- All solutions and equipment for cell culture must be sterile. All work should be performed in a biological safety cabinet or laminar flow hood to provide aseptic conditions and to prevent contamination of the cells.
- Tumor Specialized Media with 2% FBS is recommended for the majority of melanoma cell lines. The composition of Tumor Specialized Media is shown in Table 1.

  For information on exceptions to Tumor Specialized media with 2% FBS, refer to cell line specific instructions. While not preferred, DMEM with 5% FBS or RPMI with 5% FBS may be used in lieu of Tumor Specialized Media.

- Cells should be maintained between 30-95% confluence in Tumor Specialized Media with 2% FBS (heat inactivated) unless otherwise noted in cell line specific instructions.
- Media should be changed every 3-4 days. Cells should be split in a ratio according to the cell line specifications.

Table 1

Preparation of Tumor Specialized Media		
Composition	**Final Concentration**	**Volume (510 mL)**
MCDB153	**80%**	**400 mL**
Leibovitz's L-15	**20%**	**100 mL**
Fetal Bovine Serum	**2%***	**10 mL**
$CaCl_2$	**1.68 mM**	**0.42 mL**

**For information on exceptions to Tumor Specialized Media with 2% FBS, refer to cell line specific instructions.*

Splitting or Harvesting Cells

- Detach adherent cells using a dissociation reagent 0.25% trypsin/EDTA solution. The specific volumes of dissociation reagent, quench media, and Tumor Specialized Media by flask size are shown in Table 2. The incubation time with 0.25% trypsin/EDTA solution may vary; check for dissociation every 30 seconds. Observe the cells under the microscope for detachment, and when ≥ 90% of the cells have detached, extract the cells from the flask with a serological pipette.

- Transfer the cells to a 15 mL conical tube, add quench media (10% DMEM) to the cells (See Table 2), and centrifuge at 1500 rpm for 5 minutes at room temperature. Resuspend the cell pellet in Tumor Specialized media with FBS and remove a sample for counting.

- Determine the total number of cells and percent viability using a hemacytometer. Dilute cell suspension to the seeding density recommended for the cell line. Pipette the appropriate volume of cell suspension into new cell culture flask, add required volume of Tumor Specialized Media with FBS according to flask size as noted in Table 2. Return the cells to the incubator.

Table 2

Specific volumes required by flask size			
Flask size	**0.25% Trypsin/EDTA (mL)**	**Quench Medium (10% DMEM) (mL)**	**Tumor Specialized Medium with FBS (mL)**
T-25	1.0	5	3-5
T-75	3.0	10	10-12
T150	5.0	15	20-22

Freezing and Storage of Cells

The best method for cryopreserving cells is to store cultures in liquid nitrogen in complete medium in the presence of a cryoprotective agent such as dimethylsulfoxide (DMSO). Cryoprotective agents reduce the freezing point of the medium and also allow a slower cooling rate, greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death.

To freeze the cells follow the process for dislodging the cells using trypsin/EDTA solution as described above. Freezing media is composed of 90% FBS and 10% DMSO.

- Prepare freezing Media and store at 2°C to 8°C until use.
- Gently detach the cells from tissue culture flask following the trypsinization procedure as described above.
- Centrifuge cell suspension at 1500 rpm for 5 minutes at room temperature. Aseptically decant supernatant without disturbing the cell pellet.
- Resuspend the cell pellet in cold freezing Media (90% FBS/10% DMSO solution) at a density of 1 x 10^6 cells/mL. Immediately aliquot the cell suspension into cryogenic storage vials.
- Freeze the cells slowly by decreasing the temperature at approximately 1°C per minute using a controlled rate cryo-freezer or a cryo-freezing container or equivalent.
- Place the cryo-freezing container at -80°C overnight.
- Transfer cryogenic vials containing the frozen cells to liquid nitrogen, for storage in the gas phase above the liquid nitrogen.

Special Considerations

- It is important to keep track of cell line passage number as this is a crucial variable that will likely affect experimental data.
- Cell lines that are at a passage number less than 20, must be maintained at greater than 60% confluence.
- Cell lines that adhere loosely, form spheres, or tend to float can be made more adherent with the addition of serum up to 5-10%. *See the subculturing conditions in cell line specifications.*
- Cell lines should be tested for mycoplasma contamination and Short Tandem Repeat (STR) profiling every 10 passages or each time a frozen seed stock is made. *Please contact Rockland for Information regarding STR profiling analysis.*

Related Products

Product Description	Link
Genomic DNA	View Products
Total RNA	View Products
Non-viable cell pellets	View Products
Fetal Bovine Serum	FBS-01-0100

Cell lines	Stage	notes	growth
1205 Lu	Xenograft MET to WM793	xenograft MET to WM793	1:10 <1 WK
451Lu BR	BRAF Resistant	1 uM PLX	
451Lu	Xenograft MET to WM164	floats easily, grows in lightly adherent spheres	1:10 <1 WK
LH6215	MET	mesenchymal	1:4 1 wk
M331	BRAIN MET	slow	max 1:3 split
ND238	MET	melanocytic morphology	1:4 1 wk
RL159	MET	melanocytic, form attached clumps	1:4 1 WK
SKMEL28			
SKMEL28 BR	BRAF resistant	1 uM PLX	
TH202		requires 10%	1:2 2 wks
WM1026	LN MET	y, pigmented clumpy, forms spheres easily, attached cells dendritic	1:3 1.5 wk
WM115	VGP	VGP to WM239A, WM266-4 , WM165-1 Mets	1:3 1 wk
WM1158	MET	mesenchymal	1:6 <1wk
WM1232	LN MET		1:3 1 wk
WM1341D	VGP	epithelial square and triangle	1:3 1.5 wk
WM1361A	VGP	small mesenchy	1:4 1 WK
WM1366	VGP	epithelial morphology	1:10 6 days
WM1552C	RGP		1:5 1 wk
WM164	MET	Match to 451Lu	1:10 <1 WK
WM165-1	MET	fibroblastic	1:4 1 wk
WM1716	MET		
WM1727A	MET	float easily, lightly pigmented, melanocytic morphology	1:3 4 days
WM1789	UNKNOWN	thaws poorly, thin elongated fibroblastic, 1:2 2 wks in 2%, 1:3 <1wk in 10%	1:3 6 days
WM1791C	MET		1:6 4 days
WM1799	MET		1:10 <1 WK
WM1819	UNKNOWN	mesenchymal	1:6 5 days
WM1852	MET	y, NEEDS 10% to adhere, mesenchymal and spherical	
WM1862	RGP	mesenchymal and spherical, pigmented	1:4 <1wk
WM1960	MET	same patient as WM2013, WM3060	1:3 2 wks

WM1963	unknown	melanocytic	1:5 1 wk
WM1985	UNKNOWN	flat cells, tight colonies (multi-nucleated?)	1:6 1 wk
WM2013	MET	same patient as WM1960, WM3060, melanocytic	1:4 6 days
WM2032	LN MET	same patient as WM3000, flat	1:4 1 wk
WM2044	MET	y, requires 5% FBS, mesenchymal	1:6 1 wk
WM209	MET	flat tight colonies	1:6 4 days
WM2090	MET		1:6 5 days
WM239A	MET	thin elongated, fibroblastic, Met to WM115 primary	1:4 <1wk
WM262	MET	small flat cells	1:6 <1 wk
WM266-4	MET	small flat mesenchymal, Met to WM115 primary	1:3 1 wk
WM278	VGP		
WM3000	MET	another met from WM2032 patient, melanocytic	1:6 1 wk
WM3060	MET	recurrent lesion, match WM2013, WM1960, clumpy attached spheres, float easily after spilt	1:3 5 days
WM3211	VGP	fibroblastic, networks	1:3 <1 wk
WM3246	MET	large flat cells	1:4 1 wk
WM3248	unknown		1:4 <1 wk
WM3268	VGP	mesenchymal	1:4 1.5 wk
WM3282	VGP	pigmented, mesenchymal	1:3 1 wk
WM3301	MET	thaws poorly, requires at least 5%FBS, small flat	1:3 2 wks
WM3311	MET		1:3 1 wk
WM3406	MET	mesenchymal	
WM3438	MET	mesenchymal	1:3 2 wks
WM3451	LN MET	y, match WM3456 patient, thin elongated	1:4 1 wk
WM3456	MET	y, same patient as WM3451, thin elongated	1:4 2 wks
WM3482	MET	mesenchymal	1:4 2 wks
WM35	RGP		1:5 1 wk
WM3506	MET	mesenchymal	1:4 1 wk
WM3533	LN MET	fibroblastic	1:4 2wks
WM3540	LN MET	mesenchymal, pigmented	1:6 1 wk
WM3618F	LN MET OF UVEAL PRIMARY	MATCH TO 3772, attach in 10%, melanocytic, very slow	1:3 max split

WM3619	MET	mesenchymal	1:3 1 wk
WM3622	MET	large flat cells	1:3 1.5 wk
WM3623	LN MET		1:6 1 wk
WM3629	LN MET	mesenchymal and dendritic	1:6 1 wk
WM3630	MET	tight networks, mesenchymal and fibroblastic	1:4 1 wk
WM3670	LN MET	epithelial	1:3 1 wk
WM3682	LN MET	Y, ; MATCH TO 3702, 3758, same patient	1:6 1 wk
WM3702	CUT MET	Y, ; MATCH TO 3682, 3758, thin elongated, pigmented	1:4 1 WK
WM3704	MET	mesenchymal	1:6 1wk
WM373	MET	y, grows in tight adherent dense colonies, highly pigmented, very slow for a month after thaw, lots of cell die during thaw and splitting, do not split more than 1:2	1:2 3 wks once growing
WM3734	BRAIN MET		1:6 1 wk
WM3743	PRIMARY, POST CHEMO	mesenchymal	1:3 1 wk
WM3749	MET	y, mesenchymal and dendritic	1:3 2 wks
WM3755	MET	mesenchymal	1:4 < 1 wk
WM3758	MET	y, match WM3682, WM3702 same patient	1:13 1 wk
WM3772F	MET OF UVEAL PRIMARY	y, Match to 3618, need 5% to adhere, clumpy pigmented	1:4 1 wk
WM3792	MET	dendritic	1:6 10 days
WM3854	LN MET	mesenchymal	1:4 1 wk
WM39	VGP		1:6 <1 wk
WM3912	MET	y, thaws poorly, lots of spontaneous "senescent" cells	1:3 1 wk after established
WM3918	MET	needs 5-10% to adhere, if too confluent will spontaneously form tight spheres	
WM3928	LN MET	needs 10% to adhere, thaws as loosly attached cells/spheres	
WM4002	MET		1:6 3 days
WM46	MET	pigmented, fibroblastic networks	1:10 <1 wk
WM47	MET		1:6 1 wk
WM51	MET	mixed morphology, pigmented, keep culture subconfluent	1:3 1 WK
WM75	MET	pigmented	1:6 2-3 wks

WM793	VGP	match to xenograft met 1205Lu	1:10 <1wk
WM8	MET	thaws poorly	1:3 1.5 wk after established
WM852	MET	y, epithelial	1:5 1 wk
WM853-2	VGP		1:6 1 wk
WM858	LN MET	same patient as WM873	1:6 1 wk
WM873-1	LN MET	slower than -2, same patient as WM858	1:6 2 wk
WM873-2	MET	flat mesenchymal, same patient as WM858	1:6 1 wk
WM88	MET	y, melanocytic	1:5 4 days
WM9	MET	float easily, form adherent spheres, dense	1:5 1-2 wk
WM902B	VGP		1:6 1 wk
WM983A	VGP	WM983 A, B, C from same patient	
WM983B	MET	fast, loosely attached cells, spheres; WM983 A, B, C from same patient	1:10 <1wk
WM983B BR	BRAF Resistant	1 uM PLX	
WM983C	MET	WM983 A, B, C from same patient	1:6 1 wk
WM989	UNKNOWN		1:10 <1 wk