

Materials List for

In Vitro Evaluation of Oncogenic Transformation in Human Mammary Epithelial Cells

Joan Repullés^{1,2}, Mariona Terradas^{1,3,4}, Gemma Fuster^{5,6,7}, Anna Genescà¹, Teresa Anglada¹

¹Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona ²Optical Microscopy Core Facilities, IDIBELL ³Hereditary Cancer Program, Catalan Institute of Oncology, IDIBELL ⁴Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL ⁵New Therapeutic Strategies in Cancer Group. Department of Biochemistry and Molecular Biomedicine, School of Biology, Institute of Biomedicine, University of Barcelona (IBUB) ⁶Department of Biochemistry & Physiology, School of Pharmacy and Food Sciences, University of Barcelona ⁷Department of Biosciences, Faculty of Sciences and Technology, University of Vic

Corresponding Authors

Anna Genescà

Anna.Genescà@uab.cat

Teresa Anglada

Teresa.Anglada@uab.cat

Citation

Repullés, J., Terradas, M., Fuster, G., Genescà, A., Anglada, T. In Vitro Evaluation of Oncogenic Transformation in Human Mammary Epithelial Cells. *J. Vis. Exp.* (), e61716, doi:10.3791/61716 (2020).

Date Published

September 24, 2020

DOI

10.3791/61716

URL

jove.com/video/61716

Materials

Name	Company	Catalog Number	Comments
1 ml Serological Pipettes	Labclinics	PLC91001	
1.5 ml Eppendorfs	Thermo Fisher Scientific	3451	Dark eppendorfs are preferred for MTT long-term storage
10 µl Pipette tips w/o filter	Biologix	20-0010	
100 ml glass bottle			With cap, autoclavable
1000 µl Pipette tips w/ filter	Labclinics	LAB1000ULFNL	
1000 µl Pipette tips w/o filter	Biologix	20-1000	
15 ml Conical tubes	VWR	525-0400	
2 ml Serological Pipettes	Labclinics	PLC91002	
200 µl Pipette tips w/ filter	Labclinics	FTR200-96	
5 ml Serological Pipettes	Labclinics	PLC91005	
50 ml Conical Tubes	VWR	525-0304	
Acetone	PanReac AppliChem	211007	Used for 3D structure fixation prior to immunofluorescent labelling
Agar	Sigma-Aldrich	A1296	Used for anchorage assay
Anti-Claudin 4 antibody	Abcam	15104, RRID:AB_301650	Working dilution 1:100, host: rabbit
Anti-Cytokeratin 14 [RCK107] antibody	Abcam	9220, RRID:AB_307087	Working dilution 1:100, host: mouse
Anti-mouse Cyanine Cy3 antibody	Jackson ImmunoResearch Inc.	115-165-146, RRID:AB_2338690	Working dilution 1:500, host: goat
Anti-rabbit Alexa Fluor 488 antibody	Thermo Fisher Scientific	A-11034, RRID:AB_2576217	Working dilution 1:500, host: goat
Autoclave			
BioVoxel Toolbox		RRID:SCR_015825	
Cell culture 24-well Plate	Labclinics	PLC30024	Used for 3D cultures in Matrigel. Flat Bottom
Cell culture 6-well Plate	Labclinics	PLC30006	Used for anchorage assay
Cell incubator (37 °C and 5 % CO2)			

Cell Strainers	Fisherbrand	11587522	Mesh size: 40 µm
CellSense software	Olympus		Used to image acquisition
Centrifuge			
Cholera Toxin from <i>Vibrio cholerae</i>	Sigma-Aldrich	C8052	Used to supplement cell culture medium
Class II Biological Safety Cabinet	HeraSafe	HAEREUS HS12	
Confocal inverted Microscope	Leica	TCS SP5	
Cover glasses	Witeg Labortechnik GmbH	4600122	22 X 22 mm, thickness 0.13 - 0.17 mm
DAPI			2-(4-aminophenyl)-1H-indole-6-carboxamide
Fetal Bovine Serum	Biowest	S1810	Used to inactivate trypsin action
Fiji software (ImageJ)	National Institutes of Health	RRID:SCR_002285	Free download, no license needed
Glass Pasteur Pipettes			
Glass slides	Fisherbrand	11844782	
Goat Serum	Biowest	S2000	Used for immunofluorescence of 3D structures
Heat-Resistant Gloves			Used for agar manipulation after autoclave
Heater bath (37 °C)			Used to temper solutions prior to cell subculture
Heater bath (42 °C)			Used to keep agar warm
Heating plate			Used for Matrigel dehydration
Humid chamber			Used for the incubation of antibodies during immunofluorescence
Ice			Used during Matrigel manipulation
Ice-box			
Inverted Optic Microscope	Olympus	IX71	
Matrigel Matrix	Becton Dickinson	354234	Store at -20 °C and keep cold when in use. Referred to as basement membrane matrix
Methanol	PanReac AppliChem	131091	Used for 3D structure fixation prior to immunofluorescent labelling
Micropipette			p1000, p200 and p10
Microsoft Office Excel	Microsoft	RRID:SCR_016137	Used to calculate population doubling and to obtain growth rate equation
MilliQ water			Referred to as ultrapure water
Nail Polish			Used to seal samples after mounting
Parafilm M	Bemis	PM-999	Used to cover antibody solution during incubation
PBS pH 7.4 (w/o calcium & magnesium)	Gibco	10010-056	Sterile. Used for cell subculture
PBS tablets	Sigma-Aldrich	P4417	Dilute in milliQ water. No sterility required. Used for immunofluorescence
Pipette Aid			
Primaria T25 flasks	Corning	353808	Used for BPEC culture
Scepter Automated Cell Counter	Millipore	PHCC20060	Alternatively, use an haemocytometer
Scissors			Used to cut pipette tips and parafilm
Sterile filters 0.22 µm	Millipore	SLGP033RS	Used to filter MTT solution
Thiazolyl Blue Tetrazolium Bromide (MTT)	Sigma-Aldrich	M2128	Store at -20 °C

Triton X-100	Sigma-Aldrich	T8787	Used for immunofluorescence of 3D structures
Trypsin-EDTA 10X	Biowest	X0930	Dilute in PBS to obtain 3X solution
Vectashield Antifade Mounting Medium	Vector Laboratories	H-1000	
WIT-P-NC Culture Medium	Stemgent	00-0051	Used for primary BPEC culture
WIT-T Culture Medium	Stemgent	00-0047	Used for transformed BPEC culture