

Materials List for:

Establishment of Cancer Stem Cell Cultures from Human Conventional Osteosarcoma

Gaia Palmmini¹, Roberto Zonefrati¹, Carmelo Mavilia¹, Alessandra Aldinucci², Ettore Luzi¹, Francesca Marini¹, Alessandro Franchi¹, Rodolfo Capanna³, Annalisa Tanini¹, Maria Luisa Brandi¹

¹Department of Surgery and Translational Medicine (DCMT), University of Florence

²Neurofarba Department, University of Florence

³Department of Traumatology and General Orthopedics, Azienda Ospedaliera Universitaria Careggi

Correspondence to: Maria Luisa Brandi at marialuisa.brandi@unifi.it

URL: <https://www.jove.com/video/53884>

DOI: [doi:10.3791/53884](https://doi.org/10.3791/53884)

Materials

Name	Company	Catalog Number	Comments
Dulbecco's Phosphate Buffered Saline with Ca ²⁺ and Mg ²⁺ (DPBS)	LONZA	BE17-513F	–
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (DPBS)	LONZA	BE17-512F	–
Porcine Trypsin 1:250	BD Difco	215310	Solvent: DPBS. Stock concentration: Powder
Ethylenediamine tetraacetic acid disodium salt dihydrate(EDTA)	Sigma-Aldrich	E4884	Solvent: DPBS. Stock concentration: Powder
Collagenase from Clostridium histolyticum	Sigma-Aldrich	C0130	Solvent: Buffer Solution, pH 7.4. Stock concentration: Powder
Dimethyl sulphoxide (DMSO)	BDH Chemicals-VWR	10323	–
Nutrient Mixture F-12 Ham	Sigma-Aldrich	F6636	Solvent: Ultrapure dH ₂ O. Stock concentration: Powder
2-Phospho-L-ascorbic acid trisodium salt	Sigma-Aldrich	49752	Solvent: DPBS. Stock concentration: 5 mg/mL
β-Glycerol phosphate disodium salt pentahydrate	Sigma-Aldrich	50020	Solvent: DPBS. Stock concentration: 1 M
Insulin. Human Recombinant	Sigma-Aldrich	91077	Solvent: NaOH 0.1 M. Stock concentration: 10 mM
3-Isobutyl-1-methylxanthine	Sigma-Aldrich	I5879	Solvent: DMSO. Stock concentration: 500 mM
Indomethacin	Sigma-Aldrich	I7378	Solvent: DMSO. Stock concentration: 200 mM
Dexamethasone	Sigma-Aldrich	D4902	Solvent: DMSO. Stock concentration: 1 mM / 100 μM. Store in liquid nitrogen to maintain the biological activity
Fetal Bovine Serum (FBS)	Sigma-Aldrich	F7524	–
Fetal Bovine Serum South America	EUROCLONE	ECS0180L	–
Penicillin-Streptomycin (PEN-STREP) 10,000 U/mL	LONZA	DE17-602E	–
Methyl cellulose	Sigma-Aldrich	274429	Solvent: Ultrapure dH ₂ O. Stock concentration: 2%
Putresceine dihydrochloride	Sigma-Aldrich	P5780	Solvent: Ultrapure dH ₂ O. Stock concentration: Powder

apo-Transferrin	Sigma-Aldrich	T-1147	Solvent: DPBS. Stock concentration: 25 mg/mL
Human Epidermal Growth Factor (EGF)	Sigma-Aldrich	E5036	Solvent: DPBS pH 7.4. Stock concentration: 10 µg/mL
Fibroblast Growth Factor-Basic Human	Sigma-Aldrich	F0291	Solvent: DPBS + 0.2% BSA. Stock concentration: 25 µg/mL
Selenous Acid	Sigma-Aldrich	211176	Solvent: DPBS. Stock concentration: 30 mM
Progesterone	Sigma-Aldrich	P8783	Solvent: ETOH. Stock concentration: 10 mM
Toluidine Blue O	Sigma-Aldrich	198161	Solvent: Ultrapure dH ₂ O. Stock concentration: Powder
Oil Red O	ICN Biochemicals	155984	Solvent: 2-Propanol. Stock concentration: Powder
Naphtol AS-MX Phosphate Disodium Salt	Sigma-Aldrich	N5000	Solvent: DMSO. Stock concentration: Powder
Fast Blue BB Salt	Sigma-Aldrich	F3378	Solvent: Tris HCL, pH 9.0. Stock concentration: Powder
Fast Red Violet LB Salt	Sigma-Aldrich	F3381	Solvent: Tris HCL, pH 9.1. Stock concentration: Powder
Bovine Serum Albumin, Fraction V (BSA)	Sigma-Aldrich	A-4503	Solvent: DPBS. Stock concentration: 2%
Alizarin Red S	ICN Biochemicals	100375	Solvent: Ultrapure dH ₂ O. Stock concentration: Powder
Formaldehyde solution	Sigma-Aldrich	533998	4%
Triton-100X	MERCK	11869	Solvent: DPBS. Stock concentration: 0.2%. Danger - Use only under chemical hood
Calcein	MERCK	2315	Solvent: DPBS . Stock concentration: 200 µg/mL
2-Propanol	MERCK	109634	Danger - Use only under chemical hood
Ab-CD105 (Mouse monoclonal [SN6] to CD105 (FITC))	Abcam	ab11415	Liquid. Application: Flow Cytometry (Flow Cyt)
Ab-CD44 (Mouse monoclonal [F10-44-2] to CD44 (PE/Cy7®))	Abcam	ab46793	Liquid. Application: Flow Cytometry (Flow Cyt)
Ab-CD45 (Mouse monoclonal [MEM-28] to CD45 (PerCP))	Abcam	ab65952	Liquid. Application: Flow Cytometry (Flow Cyt)
Ab-CD105 (Human CD105 Purified Antibody)	Invitrogen	MHCD10500	Solvent: DPBS. Stock concentration: Lyophilized. Application: Immunofluorescence staining (IF)
Ab-CD44 (Anti-CD44 Antibody)	Abcam	EPR1013Y(ab51037)	Solvent: DPBS. Stock concentration: Lyophilized. Application: Immunofluorescence staining (IF)
Ab-Stro-1 (Mouse anti-STRO-1)	Invitrogen	398401	Solvent: DPBS. Stock concentration: Lyophilized. Application: Flow Cytometry (Flow Cyt) Immunofluorescence staining (IF)

Alexa Fluor 488 (Anti-Rabbit IgG (Alexa Fluor 488 Donkey Anti-Rabbit IgG (H+L)))	Invitrogen	A-21206	Solvent: DPBS. Stock concentration: Lyophilized. Application: Immunofluorescence staining (IF)
FITC Anti-Mouse IG (FITC-Rabbit Anti Mouse IgG (H+L))	Invitrogen	61-6511	Solvent: DPBS. Stock concentration: Lyophilized. Application: Flow Cytometry (Flow Cyt) Immunofluorescence staining (IF)
Alexa Fluor 635 Phalloidin	Invitrogen	A34054	Solvent: DPBS. Stock concentration: Lyophilized. Application: Immunofluorescence staining (IF)
AutoMACS™Running Buffer MACS Separation Buffer	Miltenyi	130,091,221	Liquid. Store at 4 °C
QIAzol®Lysis Reagent	QIAGEN	79306	Danger - Use only under chemical hood
QUANTITECT® Reverse Transcription Kit	QIAGEN	205314	–
Chlorophorm	Sigma-Aldrich	C2432	Liquid. Danger - Use only under chemical hood
Laminar flow hood	GELAIRE	BSB6A	–
Chemical hood	ARREDI TECNICI Villa	Modello DYNAMICA	–
Centrifuge	EPPENDORF	5415R	–
Laser Scanning Confocal Microscopy LSM 5109 Meta	ZEISS	–	–
iCycler PCR Thermalcycler	BIORAD	–	–
CyFlow®SPACE	(PARTEC)	–	–
Inverted Microscope Axiovert 200M	ZEISS	–	–
Freezing container ,	Nalgene	–	–
Original Pipet-Aid	pbiBrand	–	–
Micropipettes	EPPENDORF	–	–
Glass Pasteur Pipette	SIGMA	–	–
VICTOR3™	PERKIN ELMER	–	–
Conical tubes (15 and 50 mL)	BD FALCON	352096 (for 15 mL) 352070 (for 50 mL)	–
24-Well Clear Flat Bottom TC-Treated Multiwell-Cell-Culture-Plate	BD FALCON	353047	–
6-Well Clear Flat Bottom Ultra Low Attachment Multiple-Well-Plates	CORNING	3471	–
Serological pipettes (5 and 10 mL)	BD FALCON	357543 (for 5 mL) 357551 (for 10mL)	–
Syringe (5mL)	BJBRAUN	4617053V	–
Petri dish 100X20 mm	BD FALCON	353003	–
Röhren Tubes (3.5 mL, 55x12mm, PS)	SARSTEDT	55,484	–
Petri dish 60X15 mm	BD FALCON	353004	–
Cryovials 1.5 mL	NALGENE	5000-1020	–
Cell-Culture Flasks 25 cm²	BD FALCON	353014	–
Nylon Net Filter, Hydrophilic	MERCK	NY4104700	–

Swinnex Filter Holder	MERCK	SX0002500	-
Perry tweezer	-	-	-
Lancet	-	-	-
Dounce	-	-	-