

Materials List for:

Isolation and Immortalization of Patient-derived Cell Lines from Muscle Biopsy for Disease Modeling

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Materials

Name	Company	Catalog Number	Comments
Equipment			
Tissue culture biosafety hood	Baker Company, Inc.	Model SterilGuard Hood	
Benchtop centrifuge, such as Beckman Model	Beckman Coulter	Model Allegra 6R	If cell sorting is performed, a centrifuge with refrigeration is preferred
Microscope	Nikon	Model Eclipse TS100	
Tissue culture incubator, connected to a CO ₂ source	Forma Scientific	Series II	
Fluorescence-activated cell sorter (FACS)	Becton Dickinson	Model Aria	
Reagents for isolation of primary myoblasts from tissue			
Dispase II	Roche Applied Science	#04942078001	Prepare a stock solution of 2.4U/ml in DMEM
Collagenase D	Roche Applied Science	#088882001	Prepare a stock solution of 10 mg/ml
1x Sterile Hank's Balanced Saline Solution (HBSS), calcium and magnesium free	GIBCO Life Technologies	#14185-052	
Bovine serum albumin, fraction V	Sigma	#05470	Prepare a sterile solution of 1X HBSS 0.5% BSA for FACS sorting
Sterile growth medium for myoblasts: Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (4.5 g) supplemented with 30% fetal bovine serum	GIBCO Life Technologies	11965-092	Contains L- glutamine
RBC lysis solution	Qiagen	158904	
Propidium Iodide stock 10mg/ml	Sigma	P4170	Prepare the stock solution by diluting the powder in sterile distilled water
Anti-human CD56 antibody for flow cytometry	Biolegend	318310	APC-conjugated antibody, other labels are available
Reagents for immortalization of primary myoblasts			
Ecotropic packaging cell line PE	Cell Biolabs	RV-101	
Amphotropic packaging cell line PA3174	Cell Biolabs	RV-102	

Pig skin gelatin	Sigma	G1890-500g	Prepare a stock solution of 0.1% gelatin in water. Coat the dish with the solution at 37°C for one hour. Remove the solution and add medium.
PolyJet	Signagen	SL 100688	
G418	Fisher	345812	
Hygromycin	EMD Biosciences	400051	
pBabe plasmids containing mCDK4 and hTERT	not commercially available		Stadler et al, 2013
Qiagen plasmid midiprep kit	Qiagen	12143	
Medium 199	Life Technologies	Medium 199 (31150022)	
Dulbecco's modified Eagle medium (DMEM)	Life Technologies	DMEM (11965-092)	Mix 4:1 DMEM:199
Myoblast growth medium: 4:1 Dulbecco's modified Eagle medium (DMEM) and Medium 199 supplemented with 15% fetal bovine serum; 0.02 M HEPES buffer; 1.4 mg/L vitamin B12; 0.03 mg/L ZnSO ₄ , 0.055 mg/L dexamethasone, 2.5 µg/L hepatocyte growth factor and 10 µg/L beta fibroblast growth factor.	Life technologies (DMEM, F199); Atlanta Biological (FBS); Invitrogen (Hepes); Fisher (ZincSulfate); Sigma (Vit.B12, Dexamethasone); Chemicon international (HGF); Biopioneer (betaFGF)	#15630-080 (Hepes); #Z68-500 (ZnSO ₄); #V2876.#D4902 (Vit.B12, Dex); GF116 (HGF); HRP-0011 (bFGF).	Prepare media and stock solution Vit. B12 (20mg/ml); ZincSulfate (60µg/ml); Dex (55µg/µl) separately for easier use. HGF stock solution (5µg/ml) and FGF (20µg/ml) should be added freshly every week at the final working concentration.
TrypLE express	GIBCO Life Technologies	12605-010	
Myosin heavy chain antibody for immunostaining	Developmental Hybridoma Bank	MF20	Clone MF20
Desmin Antibody for immunostaining	Thermo Scientific	MS-376-S0	Clone D33
Horse serum	Invitrogen	26050-088	
Reagents for primary skin fibroblast isolation			
Transport medium: RPMI 1640 supplemented with 10% fetal bovine serum and 0.2% penicillin/streptomycin	RPMI medium 1640: GIBCO Life Technologies	11875-093	
Human primary fibroblast culture medium: RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin	Fetal bovine serum: Thermo Scientific	SH30071.03	
Collagenase solution: Collagenase type 2 (100 mg) resuspended in 12.5 ml of fibroblast culture medium and filter-sterilized	Worthington	4176	
Sterile 1X Phosphate Buffer Saline (PBS) calcium and magnesium free	Lonza	17-516F	
Materials for isolation of primary myoblasts			
50 ml and 15 ml sterile conical tubes	GeneMate/Bioexpress	C-3394-4 (50 ml) ; C3394-1 (15 ml))	
Sterile scalpels	Aspen Surgical	372610	
Hemocytometer	Hausser Scientific	1492	
Sterile 5, 10, and 25 ml pipettes	Bellco glass	1226-05010 (5 ml); 1200-10010 (10 ml) ;1228-25050 (25 ml)	Reusable pipettes are washed, cotton plugged and autoclaved before use

Sterile tissue culture-treated plastic dishes (10 cm)	BD Falcon	353003	
Sterile nylon cell strainers (100 μ m and 40 μ m size)	BD Falcon	352340 (40 μ m); 352360 (100 μ m)	
Materials for myoblast immortalization			
Sterile 0.45 μ m filters	Millipore	SLHV013SL	
Cloning rings	Corning	#3166-8	To be cleaned and autoclaved before and after use
Materials for fibroblast cell lines			
Sterile 35 mm tissue culture-treated plastic dishes	Greiner bio-one	628160	
Sterile scalpels	DeRoyal	D4510A	
Sterile T25 tissue culture flasks	Techno Plastic Product, TPP	90026	sold in the USA by MIDSCI
TrypLE express	GIBCO Life Technologies	12605-010	