

Materials List for:

# Isolation, Culture, and Transplantation of Muscle Satellite Cells

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URL: <https://www.jove.com/video/50846>

DOI: [doi:10.3791/50846](https://doi.org/10.3791/50846)

## Materials

Name	Company	Catalog Number	Comments
<b>Materials</b>			
Collagenase Type 2	Worthington	CLS-2	100 mg
Marigel	BD Biosciences	356234	5 ml
DMEM	Gibco-Invitrogen	10569010	500 ml
Collagen (Rat Tail)	BD Biosciences	354236	100 mg (3-4 mg/ml)
Acetic Acid	Sigma-Aldrich	320099-500ML	500 ml
bFGF, human, Recombinant	Gibco-Invitrogen	PHG0263	1 mg
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A5611-1G	1 g
Ham's F10 Medium	Gibco-Invitrogen	11550-043	500 ml
Fetal Bovine Serum (FBS)	Fisher Scientific	3600511	500 ml
Horse Serum	Gibco-Invitrogen	26050088	500 ml
Penicillin/Streptomycin	Gibco-Invitrogen	15640055	100 ml
Phosphate Buffered Saline	Gibco-Invitrogen	14190144	500 ml
0.25% Trypsin/EDTA	Gibco-Invitrogen	25200072	500 ml
18 G needle with 12 ml Syringe	Fisher Scientific	22-256-563	
Cell strainer (70 µm)	Fisher Scientific	22-363-548	
Falcon 50 ml tube	BD Biosciences	352098	
Falcon 15 ml tube	BD Biosciences	352097	
10 cm Tissue culture plate	BD Biosciences	353003	
6 cm Tissue culture plate	BD Biosciences	353004	
Falcon 10 ml disposable pipette	BD Biosciences	357551	
Anti-CD31 antibody-PE	eBiosciences	12-0311	
Anti-CD45 antibody-PE	eBiosciences	30-F11	
Anti-Sca1 antibody-PE	eBiosciences	Dec-81	
Anti-Integrin α7 antibody	MBL International	ABIN487462	
Anti-PE MicroBeads	Miltenyi Biotec	130-048-801	
Anti-Mouse IgG MicroBeads	Miltenyi Biotec	130-048-402	
Mini & MidiMACS Starting Kit	Miltenyi Biotec	130-091-632	
MS Column	Miltenyi Biotec	130-042-201	
LD Column	Miltenyi Biotec	130-042-901	
Cardiotoxin	Sigma Aldrich	C9759-1MG	Stock 10 µM in PBS
31 G Insulin syringe	BD Biosciences	328438	
Refrigerated Microcentrifuge (Microfuge 22R)	Beckman Coulter	368826	
S241.5 Swinging Bucket Rotor	Beckman Coulter	368882	

Refrigerated Centrifuge (Allegra X-22R)	Beckman Coulter	392187	
Nod/Scid immunodeficient mice	Charles River Laboratories	Strain Code 394	Use 2 months old mice
<b>Reagents</b>			<b>Recipe</b>
10% and 2% FBS DMEM			DMEM (Gibco-Invitrogen #10569010) with 10% or 2% FBS (Fisher Scientific #03600511) and 1% Penicillin/Streptomycin (Gibco-Invitrogen #15640055).
0.2% Collagenase solution			Collagenase Type 2 (Worthington, #CLS-2), Stock: 50 ml: 100 mg Collagenase Type 2 in 10% FBS DMEM.
10% Matrigel solution			Matrigel (BD Biosciences: #356234) is placed on ice for thawing overnight. Five ml Matrigel is dilute by 45 ml DMEM and 5 ml aliquots are stored at -20 °C until use.
Matrigel-coated plate			Five ml of 10% Matrigel solution is placed on ice for thawing and is used for coating 10 cm plate at room temperature for 1 min. The plate is placed in 5% CO <sub>2</sub> incubator at 37 °C for 30 min after removing Matrigel solution, and let the plate dry in culture hood for another 30 min. Removed 10% Matrigel solution is stored at -20 °C for reuse.
0.01% Collagen solution			Mix to final: 0.01% Collagen (Collagen, Rat Tail: BD Biosciences #354236) in 0.2% acetic acid (320099-500ML) in ddH <sub>2</sub> O.
Collagen-coated plate			Add 5 ml or 2 ml of Collagen solution to a 10 cm or 6 cm tissue culture plate and let sit at room temperature for three hours. Then, aspirate off liquid and allow to dry in culture hood for 30 min to overnight. Plates can be stored at room temperature for several months.
bFGF stock solution			bFGF, Human, Recombinant (Gibco-Invitrogen #PHG0263, 1 mg) is dissolved with 0.1% BSA solution consisting of 1 mg BSA (Sigma-Aldrich #A5611-1G) and 2 ml ddH <sub>2</sub> O (0.5 mg/ml bFGF). Aliquot 20 µl in 500 µl microcentrifuge tubes and kept in -80 °C.
Myoblast medium			500 ml HAM'S F10 Medium (Gibco-Invitrogen #11550-043) supplemented with 20% FBS (Fisher Scientific #03600511), Penicillin/streptomycin (Gibco-Invitrogen #15640055), and 10 µg of bFGF (20 µl of bFGF stock).
Differentiation medium			500 ml DMEM (Gibco-Invitrogen #10569010) supplemented with 5% Horse serum (Gibco-Invitrogen

			#26050088) and 1% Penicillin/streptomycin (Gibco-Invitrogen #15640055).
10 $\mu$ M Cardiotoxin stock			1 mg Cardiotoxin (EMD Millipore #217504-1MG) is dissolved with 13.9 ml PBS.