

Materials List for:

# Preparation of Primary Myogenic Precursor Cell/Myoblast Cultures from Basal Vertebrate Lineages

Jacob Michael Froehlich<sup>1</sup>, Iban Seilliez<sup>2</sup>, Jean-Charles Gabillard<sup>3</sup>, Peggy R. Biga<sup>1</sup>

<sup>1</sup>Department of Biology, University of Alabama at Birmingham

<sup>2</sup>Nutrition Métabolisme Aquaculture, INRA UR1067

<sup>3</sup>Laboratoire de Physiologie et Genomique des Poissons, INRA UR1037

Correspondence to: Peggy R. Biga at [pegbiga@uab.edu](mailto:pegbiga@uab.edu)

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## Materials

Name	Company	Catalog Number	Comments
Table 1. Detailed Reagent Information			
Reagent	Company (Preferred v. Alternate)	Catalog Number (Preferred v. Alternate)	Quantity per Culture
γ-irradiated poly-L-lysine	Sigma-Aldrich (MP Biomedicals)	P5899 (ICN19454405)	5 mg
DMEM (high glucose)	Sigma-Aldrich (cellgro)	MT-50-003-PB (D7777)	2 L
Laminin	BD Biosciences (Sigma-Aldrich)	CB-40232 (L2020)	1 mg
Sodium Bicarbonate (NaHCO <sub>3</sub> )	Fisher Scientific (Sigma-Aldrich)	BP328-500 (S5761)	1.51 g
HEPES (C <sub>8</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub> S)	Fisher Scientific (Sigma-Aldrich)	BP310-1 (H6147)	9.53 g
Antibiotic/Antimycotic	Thermo Scientific (Sigma-Aldrich)	SV3007901 (A5955)	17-20 mL
Gentamicin Sulfate	Lonza (Sigma-Aldrich)	BW17-519Z (G1397)	2-3 mL
Donor Equine Sera	Thermo Scientific (Sigma-Aldrich)	SH3007403 (H1270)	75 mL
Fetal Bovine Sera	Thermo Scientific (Sigma-Aldrich)	SH3007103 (F2442)	25 mL
Collagenase (Type IV)	Worthington (Sigma-Aldrich)	LS004189 (C9891)	0.44 g
Trypsin (from Pancreas)	MP Biomedicals (Sigma-Aldrich)	ICN15357125 (T5266)	1 g
Table 2. Consumables, Tools and Equipment			
Consumable	Tools	Equipment	
Cell Culture Plates	Forceps (Coarse)	Serological Pipettor	
Sterile 50 mL Conical Tubes	Forceps (Fine)	pH Meter	
Laboratory Tape	Scalpel Handles	Chilling Incubator (Echotherm)	
0.2 μm Vacuum Sterilization Systems	Scalpel Blades (#10, #11)	Laminar Flow Hood	
Water-repellant Autoclave Paper	Surgical Scissors	Vacuum Manifold	
Serological Pipettes	Glass Petri Dishes	Microosmolality Meter	
	12-16 G Cannulas with Luer Locks		
Table 3. Optimized Volumes for Coating Cell Culture Plates			
Plate Size	cm <sup>2</sup> per Well	Poly-L-lysine*	Laminin**
6 well	9.5	1.6 mL	1
24 well	1.9	0.32	0.2
48 well	0.95	0.16	0.1
96 well	0.32	0.06	0.03
*0.1 mg/mL concentration	** 0.020 mg/mL concentration		
Table 4. Media for Isolation, Dissociation, and Culture			
Reagent	Isolation	Wash	Dissociation

Base Medium	419.25 mL	395.40 mL	297.00 mL
Gentamicin Sulfate**	0.75 mL	0.60 mL	-
Donor Equine Serum	75.00 mL	-	-
Fetal Bovine Serum***	-	-	-
* PSF: penicillin/streptomycin/ fungizone cocktail (100x); ** 50 mg/mL concentration; *** Characterized			

Table 5. Recommended Dilutions and Plating Volumes

Plate Size	cm <sup>2</sup> per Well	Dilution	Plating Volume
6 well	9.5	1.5-2.0x10 <sup>6</sup> cells/mL	1 mL
24 well	1.9	1.5-2.0x10 <sup>6</sup> cells/mL	250 $\mu$ L
48 well	0.95	1.5-2.0x10 <sup>6</sup> cells/mL	150 $\mu$ L
96 well	0.32	1.5-2.0x10 <sup>6</sup> cells/mL	50-100 $\mu$ L

Table 6. Average number of cells per g tissue

Species	Average # cells/g tissue		
Danio rerio	6,400,000		
Danio dangila	1,783,000		
Devario aequipinnatus	1,797,000		
Oncorhynchus mykiss	66,800		

Table 7. Recommended Incubation Temperatures

Species	Temperature		
Danio/ Devario spp.	26 - 28 °C		
Oncorhynchus/Salmo spp.	10* - 18 °C		
Ambystoma mexicanum	18°C		
* Lower temperatures support lower proliferation rates.			