

Materials List for:

# Isolation and Culture of Neonatal Mouse Cardiomyocytes

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## Materials

Name	Company	Catalog Number	Comments
BDM (2,3-Butanedione monoxime) <sup>6-8</sup>	Sigma	B-0753	prepare 0.2M stock solution in HBSS (without Ca <sup>2+</sup> , Mg <sup>2+</sup> ), filter sterilize, can be kept at 4 °C up to 6 months; Caution: Prolonged usage of BDM other than during isolation procedure may result in non-beating cells, decreased cell viability and/or significantly altered gene-expression during cardiomyocyte culture <sup>46,47</sup> .
Collagenase/Dispase	Roche	10269638001	can be substituted with collagenase type II from Worthington
Collagenase type II <sup>3</sup>	Worthington	CLS-2	substitute for Collagenase/Dispase mix from Roche
1x trypsin solution (0.25%) with EDTA	cellgro	25-053-CI	
1x Penicillin/ Streptomycin solution with EDTA in HBSS	cellgro	30-002-CI	
1x PBS (without Ca <sup>2+</sup> , Mg <sup>2+</sup> )	e.g, cellgro	21-040-CV	
HBSS (Hank's balanced salt solution; without Ca <sup>2+</sup> , Mg <sup>2+</sup> ) <sup>4</sup>	cellgro	21-022-CV	
DMEM high glucose	cellgro	10-013-CV	
M-199	cellgro	10-060-CV	
fetal bovine serum	cellgro	35-011-CV	cell-culture grade
horse serum	cellgro	35-030-CV	cell-culture grade
Leibovitz L-15 <sup>5</sup>	cellgro	10-045-CV	
AraC (Cytosine-B-D-arabino-furanoside hydrochloride)	Sigma	C-6645	proliferation inhibitor, prepare 1 mM stock solution in H <sub>2</sub> O, filter sterilize, store at 4 °C
phenylephrine	Sigma	P-6126	chronotropic agent, prepare 100 mM stock solution in H <sub>2</sub> O, filter sterilize, store at 4 °C or -20 °C
isoproterenol hydrochloride	Sigma	I-6501	chronotropic agent, prepare 1 mM stock solution in H <sub>2</sub> O, filter sterilize, store at 4 °C or -20 °C
0.1% collagen solution	Sigma	C-8919	extracellular matrix for coating
3 mg/ml collagen type 1 solution	Advanced BioMatrix	5005-B	alternative to Sigma collagen solution
cell strainer	Fisherbrand	22363548	appropriate filter size:40 µm-100 µm

syringe filter 0.2 µm	Fisherbrand	09-719C	for sterile filtration of digestion medium
straight scissors	Fine Sciences Tools	91460-11	
curved scissors	Fine Science Tools	91461-11	
Dumont No. 7 forceps	Fine Science Tools	91197-00	
perforated spoon	Fine Science Tools	10370-19	optional, for transfer of heart tissue
Trypan blue	Gibco	15250-061	live cell staining
Neubauer hemocytometer	Prosource Scientific	3500	alternatively use: disposable hemocytometer C-chip or automated cell counting systems
50 ml Falcon tubes	Fisherbrand	14-432-23	
15 ml Falcon tubes	Fisherbrand	05-527-90	
20 ml syringe	BD Medical	14-820-19	
10 ml serological pipette	Falcon	357551	
30 mm cell culture dish	Nunc	153066	for standard culture of cardiomyocytes
30 mm cell culture dish, glass bottom	MatTek	P35G-0-10-C	for live cell imaging with inverted microscope
10 cm cell culture dish	Nunc	172958	for preplating
Escort III	Sigma	L3037	for liposomal transfection, alternatively use lipofectamin 2000
Lipofectamine 2000	Life Technologies, Invitrogen	52887	substitute for Escort III

**Buffers and media:**

- **Isolation medium (filter sterilize)**  
20 mM BDM  
0.0125% trypsin  
in HBSS<sup>4</sup> (without Ca<sup>2+</sup>, Mg<sup>2+</sup>)
- **Digestion medium (filter sterilize)**  
20 mM BDM  
1.5 mg/ml Roche Collagenase/Dispase enzyme mix  
in L15 medium
- **Plating medium**  
65% DMEM high glucose  
19% M-199  
10% horse serum  
5% fetal calf serum  
1% penicillin/streptomycin
- **Maintenance medium**  
78% DMEM high glucose  
17% M-199  
4% horse serum  
1% penicillin/streptomycin  
*optional:*  
1 µM AraC  
1 µM isoproterenol or 0.1 mM phenylephrine

The plating and maintenance medium can be stored at 4 °C for up to 6 months.