Materials List for:

Analysis of Trunk Neural Crest Cell Migration using a Modified Zigmond Chamber Assay

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Materials

Name	Company	Catalog Number	Comments
DMEM	Omega Scientific	DM-22	
Penicillin Streptomycin Solution	Omega Scientific	PS-20	100X Stock Concentration
L-Glutamine	Omega Scientific	GS-60	100X Stock Concentration
Fetal Bovine Serum	Omega Scientific	FB-11	Lot# 105247 (or another that is comparable)
Modified Zigmond chamber	Home made	N/A	Reservoir volume: ~ 160 µl ea; for additional specifications, see Fig. 4 and the supplemental fabrication protocol
Cell culture dish	Denville	T6040	40 x 10 mm
Fibronectin	BD	354008	10X Stock prepped by diluting 1 mg FN in 1 ml H_2O and 9 ml DMEM
Coverslips	Fisher	12-548-B	Precleaned; 22 x 22 mm
L15 medium	Thermo Scientific	SH30525.02	
Petroleum Jelly	Comforts	011110794642	100%
Centrifuge tube	Biologix	10-9152	15 ml
Dispase	Cell Systems	4Z0-850	10X Stock Concentration
Syringe	BD	309602	1 ml
Needle	BD	305127	25 G x 1.5 in.
Alexa Fluor 488-IgM	Invitrogen	A21042	Stock is 2 mg/ml; 7 moles dye/ mole IgM
Dissecting Forceps	FST	Misc.	Dumont #5 or 55; straight tipped; stainless steel or titanium
Tungsten Needle	N/A	N/A	Home made; placed in a pin holder
Blunt Forceps	Tiemann	160-18	Used for transferring embryos to Ringer's from egg yolk

Supplemental Protocol: Fabrication of a Modified Zigmond Chamber

Please refer to Figure 4 as a reference for the protocol below:

1. Purchase a sheet of 3/16" thick polished acrylic (4.45 mm actual thickness).
2. Using a table saw, cut chamber blanks oversized to the rough dimensions of 33.25 mm x 64.57 mm. This allows 3.175 mm extra material for machining.
3. Set the chamber blank on a vise. With a milling machine and a 6.35 mm (1/4") end mill bit, finish machining the sides of the chamber to their exact dimensions: 30.07 mm x 61.39 mm.
4. Position the chamber blank on the milling machine and locate the center of the blank along both the x and y axes with an edge finder; then zero the center location.
5. Acquire the chamber height (z-axis) by touching the end mill bit to the top surface and zero the height.

6. Using a 3.91 mm (0.154") end mill bit, offset the bit 3.03 mm along the x-axis (positive direction) for the first reservoir. Begin machining into the chamber to a depth of 2.84 mm while moving along the y-axis (positive direction) to 7.62 mm (0.300") and then traverse to 7.62 mm (0.300") in the opposite (negative) direction to a complete reservoir length of 15.24 mm (0.600"). Offset the bit to 3.03 mm (0.119") along the x-axis (negative direction) and repeat the same process for the second reservoir.
7. Position the chamber on its edge and drill a hole using a 1.09 mm (0.043 in.) drill bit on the end of each reservoir (4 total) that connects the end of the reservoir to the side of the chamber for loading medium during experimentation.
8. Soak the chamber well in warm soapy water to help remove any chemical contaminants.
9. Soak and rinse the chamber well in double-distilled water to remove any soap. The chambers are now ready to use as described above.

