Materials List for:

Preparation of Neuronal Cultures from Midgastrula Stage Drosophila Embryos

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Materials

Name	Type	Company	Catalog Number	Comments
Drosophila melanogaster	Animal			Fruit flies
Transferrin	Reagent	Sigma-Aldrich	T-1147	100x Stock: 10 mg/ml in water. Filter through 0.2 um syringe filter (cellulose acetate). Store in 220 ul aliquots (for 20 ml DMEM) at -20C.
Insulin		Sigma-Aldrich	I-6634	200x Stock: 10 mg/ml in 0.05N HCl. Filter through 0.2 um syringe filter (cellulose acetate). Store in 120 ul aliquots (for 20 ml DMEM) at -20C.
Putrescine		Sigma-Aldrich	P-5780	100x Stock: 10 mM in ddH2O. Filter through 0.2 um syringe filter (cellulose acetate). Store in 220 ul aliquots (for 20 ml DMEM) at -20 C
Selenium		Sigma-Aldrich	S-5261	100x Stock: 3 uM in ddH2O. Put 0.0051 g Selenium in a 15 ml tube labeled A (3 mM stock). Add 10 ml of sterile water. Take 10 ul from Tube A to Tube B with 10 ml ddH2O (3 uM stock). Filter Tube B through 0.2 um syringe filter (cellulose acetate). Store in 220 ul aliquots (for 20 ml DMEM) at -20C
Progesterone		Sigma-Aldrich	P-6149	100x Stock: 2 ug/ml1.Add 1ml of 100% EtOH to 0.001 g Progesterone bottle2.Add 49 ml ddH2O3.Transfer 1 ml of this to second tube with 9 ml ddH2O4.Filter through 0.2 um syringe filter (cellulose acetate)5.Store in 220 ul aliquots (for 20 ml DMEM) at -20C
DDM1	Medium			To 10 mls of DMEM add from stocks:100 ul Transferrin, 100 ul Putrescine,100 ul Selenium,100 ul Progesterone, 50 ul Insulin
Petri dishes				
Cover-slips		Bellco Glass	1943-00012	Low lead glass, autoclaved.

Paper filter		Whatman, GE Healthcare	#1	
10cc syringe	Tool			
The cultures made in this media must be maintained in a 5% CO2 incubator at about 22-24°C. We use a standard mammalian tissue culture incubator placed in a cold room and heated to 23°C. Always used autoclaved filtered water and make in containers that are reserved for media and supplements only. Never put a pH meter or stir bar used for other purposes in media.				