

Materials List for:

Culture of Isolated Floor Plate Tissue and Production of Conditioned Medium to Assess Functional Properties of Floor Plate-released Signals

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Materials

Name	Company	Catalog Number	Comments
Reagents Required			
PBS	Invitrogen	14190-094	
HBSS, Ca ²⁺ /Mg ²⁺ - free	Invitrogen	14170-088	
Glucose	Sigma	G-7021	
Neurobasal	Invitrogen	21103-049	
Plasma	Sigma	P-3266	1 mg/ml (Reconstitute in H ₂ O and filtrate)
Thrombin	Sigma	T-4648	10 mg/ml(Reconstitute in H ₂ O and filtrate)
B27	Invitrogen	17504-044	50x
HEPES (260 g/mol)	Sigma	H-7006	
EDTA	Sigma	E-5513	0.5 M, pH 8
MgCl ₂	Euromedex	2189	1M
Glycerol	VWR	24388.295	
IGEPAL	Sigma	I-3021	
Complete Protease Inhibitor Cocktail Tablets	Roche	04 693 116 001	
Sodium Orthovanadate (Na ₃ VO ₄)	Sigma	S-6508	
Distilled H ₂ O			

Table 1.Reagents required.

Tools and Materials

Surgical scissors	Fine Science Tools	14002-12	
Adson forceps	Fine Science Tools	11027-12	
Dumont #5 Fine Tips forceps	Fine Science Tools	11254-20	
Microknives	Fine Science Tools	10136-14	

Table 2. Tools and materials.

<i>Dissection and FP culture</i>	
Ethanol 70%	
dissection hood	
dissection microscope	
Petri dishes	
glass coverslips	18 mm x 18 mm
Bunsen gas burner	
24-well plate	
tissue culture incubator (37 °C, 5% CO ₂ , humidity controlled)	

Centrifuge	
Recipes	
<i>FP culture media</i>	
Neurobasal	
B27	1/50e dilution from stock
<i>HBSS-thrombin</i>	
HBSS	1 ml
Thrombin 10 mg/ml	30 µl
<i>Lysis Buffer pH 7,4</i>	
HEPES (260 g/mol)	25 mM
EDTA (pH 8)	5 mM
MgCl ₂	1 mM
Glycerol	10%
IGEPAL (NP40)	0.10%
Protease Inhibitor Cocktail	1x
Sodium Orthovanadate	0.2 M
H ₂ O	
<i>Laemmli Buffer</i>	
Tris 0.5 M, pH 6,8	375 mM
SDS	6%
Glycerol	60%
DTT	0.6 M
Bromophenol blue	
H ₂ O	