

A Cell Culture Model for Studying the Role of Neuron-Glia Interactions in Ischemia

Genilso Gava-Junior^{*1}, Cláudio Roque^{*1}, Julieta Mendes-Oliveira¹, Ana C Bernardino¹, Inês Serrenho¹, Joel P Pires¹, Graça Baltazar^{1,2}

¹Centro de Investigação em Ciências da Saúde (CICS-UBI), Universidade da Beira Interior ²Faculdade de Ciências da Saúde, Universidade da Beira Interior

*These authors contributed equally

Corresponding Author

Graça Baltazar

gbaltazar@fcsaude.ubi.pt

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Materials

Name	Company	Catalog Number	Comments
24 -well culture plates	Thermo Fischer Scientific	142475	
95% N ₂ /5% CO ₂ gas cylinder	ArLíquido		
Anti-mouse conjugated to Alexa Fluor 488	Invitrogen	A11001	1/1000 dilution; incubation period - 1 h at room temperature
Anti-rabbit conjugated to Alexa Fluor 546	Invitrogen	A11010	1/1000 dilution; incubation period - 1 h at room temperature
B27 supplement (50x)	Gibco	17504-044	
Dako Fluorescence Mounting Medium	Dako	S3023	
D-glucose anhydrous	Fisher Scientific	G/0450/60	3.4 g/L
Epifluorescence microscope	Zeiss	AxioObserver Z1x	63x objective
Fetal Bovine Serum (FBS)	Biochrom	S0615	10%
Gentamicin	Sigma-Aldrich	G1272	120 µg/mL
Glutamate	Sigma-Aldrich	G8415	25µM
Glutamine	Sigma-Aldrich	G3126	0.5 mM
Hoechst 33342	Invitrogen	H1399	2 µM; incubation period - 10 min at room temperature
Hypoxia incubation chamber	Stemcell Technologies	27310	Chamber used for OGD induction
Insulin from bovine pancreas	Sigma-Aldrich	I5500	5 mg/L
Ketamine	Sigma-Aldrich	K-002	87.5 mg/Kg
Minimum Essential Medium Eagle medium	Sigma-Aldrich	M0268	warm up to 37 °C before use
Mouse Anti-MAP2	Santa Cruz Biotechnology	Sc-74421	1/500 dilution; incubation period overnight at 4 °C
Neurobasal medium	Gibco	21103-049	warm up to 37 °C before use
Paraffin pastilles for histology	Sigma-Aldrich	1.07164	Solidification point 56-58°C
Paraformaldehyde	Sigma -Aldrich	P6148	4% in PBS

Penicilin/Streptomycin	Biochrom	A 2213	penicillin (12U/mL) /streptomycin (12µg/mL)
Poly-D-lysine	Sigma-Aldrich	P1024	
Rabbit Anti-GFAP	DAKO	Z0334	1/2000 dilution; incubation period overnight at 4 °C
Sodium hydrogen carbonate	Fisher Scientific	S/4240/60	2.2g/L
Xylazine	Sigma-Aldrich	X1126	12 mg/Kg