

Materials List for:

# Quantitative Analysis of Synaptic Vesicle Pool Replenishment in Cultured Cerebellar Granule Neurons using FM Dyes

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## Materials


Name	Company	Catalog Number	Comments
AxioCam MRm Rev. 3 Digital Camera	Carl Zeiss, Inc.	4265099901000	
Axio Observer.A1 Microscope	Carl Zeiss, Inc.	4310040000000	
Cell culture plates (6 wells)	Greiner Bio-One	657160	
Centrifuge (Universal 32R)	Hettich Zentrifugen	1610	
CO <sub>2</sub> incubator	Heraeus Instruments	51014042	
Falcon tubes (15/50 ml)	Greiner Bio-One	188271/210261	
Fluar 20X /0.75 ∞/0.17 Objective	Carl Zeiss, Inc.	4401459901000	
Glass coverslips (25mm)	VWR international	631-1584	
Glass pasteur pipettes (230 nm)	Greiner Bio-One	612-1799	
Haemocytometer	VWR international	15170-170	
Imaging chamber	Warner Instruments	RC-21 BRFS	
Laminar flow hood	BIOHIT	VLF BHS 1200	
Mcllwain Tissue Chopper	Mickle Laboratory Engineering Co. Ltd.	MTC/2	
Mercury lamp	Carl Zeiss, Inc.	HBO 103	
<a href="#">MultiStim System Electrical Stimulator (100mV, 1ms pluse width)</a>	 Digitimer Ltd.	<a href="#">D330</a>	
Perfusion pump	Watson-Marlow Pumps Group	313S	
Serological Pipettes (5/10/25 ml)	Greiner Bio-One	606180/607180/760180	
Shutter controller	Carl Zeiss, Inc.	MAC5000	
Syringe (20 ml)	BD Biosciences	ST01-B002	
Syringe Filters (Minisart — 0.20 µm)	Sartorius AG	16532	
VC-6 Six Channel valve controller	Warner Instruments	64-0135	
YFP Filter set (Set 46)	Carl Zeiss, Inc.	1196-681	

Table 1. Specific equipment and apparatus used

FM1-43	Cambridge BioScience	BT70021	10 µM
FM2-10	Cambridge BioScience	BT70044	100 µM
Poly-D-lysine	Sigma-Aldrich	P7886	15 µg/ml
Silicone grease	Sigma-Aldrich	85403	-

Table 2. Specific reagents used

Bovine Serum Albumin (BSA)	Sigma-Aldrich	A4503	0.3%
D-Glucose	Sigma-Aldrich	G5767	0.25%

MgSO <sub>4</sub> ·7H <sub>2</sub> O	Sigma-Aldrich	M2773	1.5 mM
D-PBS	GIBCO, by Life Technologies	21300	960 mg/100 ml
-make 100ml fresh for each preparation -sterile filter before use			
Table 3. Solution B for CGN preparation			
Solution B			19 ml
Trypsin (5 mg/ml stock, -20°C)	Sigma-Aldrich	T9201	1 ml
Table 4. Solution T for CGN preparation			
Solution C			3.2 ml
Solution B			16.8 ml
Table 5. Solution W for CGN preparation			
Deoxyribonuclease (DNase, 500 U per 0.5 ml stock, -20°C)	Sigma-Aldrich	D5025	0.5 ml
MgSO <sub>4</sub> · 7H <sub>2</sub> O	Sigma-Aldrich	M2773	1.5 mM
Solution B			10 ml
Soybean trypsin inhibitor (SBTI, 0.5 mg per 0.5 ml stock, -20°C)	Sigma-Aldrich	T9003	0.5 ml
Table 6. Solution C for CGN preparation			
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A4503	4%
Earle's Balanced Salt Solution (EBSS)	GIBCO, by Life Technologies	24010	10 ml
MgSO <sub>4</sub> · 7H <sub>2</sub> O	Sigma-Aldrich	M2773	3 mM
Table 7. EBSS Solution for CGN preparation			
Cytosine β-D-arabinofuranoside (Ara-C) *	Sigma-Aldrich	C1768	10 μM
F-tal Bovine Serum	GIBCO, by Life Technologies	10106	10 %
D-Glucose	Sigma-Aldrich	G5767	30 mM
L-Glutamine	Sigma-Aldrich	G3126	2 mM
KCl	Sigma-Aldrich	P5405	25 mM
Minimal Essential Medium (MEM)	GIBCO, by Life Technologies	21090	500 ml
Penicillin (P)/Streptomycin (S)	GIBCO, by Life Technologies	15140	100 U/ml (P), 100 μg/ml (S)
*Ara-C should be added to medium from 1 DIV onwards			
Table 8. Culture Media for CGN preparation			
AxioVision Rel.	Carl Zeiss, Inc.	4.8	
ImageJ	National Institutes of Health	1.42q	
Microsoft Excel	Microsoft	2003	
Table 9. Specific computer software used			
CaCl <sub>2</sub> · 2H <sub>2</sub> O	Sigma-Aldrich	C7902	1.3 mM
Glucose	Sigma-Aldrich	G5767	5 mM
KCl	Sigma-Aldrich	P5405	3.5 mM
KH <sub>2</sub> PO <sub>4</sub>	Sigma-Aldrich	P9791	0.4 mM
MgCl <sub>2</sub> ·6H <sub>2</sub> O	Sigma-Aldrich	M0250	1.2 mM
NaCl	Fluka	71378	170 mM
NaHCO <sub>3</sub>	Fluka	71627	5 mM
Na <sub>2</sub> SO <sub>4</sub>	VWR	10264	1.2 mM
TES	Sigma-Aldrich	T1375	20 mM

Table 10. Saline Solution (pH 7.4)