

Materials List for:

Methods for the Discovery of Novel Compounds Modulating a Gamma-Aminobutyric Acid Receptor Type A Neurotransmission

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Materials

Name	Company	Catalog Number	Comments
General equipment			
96 well cell harvester -(Filtermate 196)	Packard		To filter membranes with bound radioligand
Microtiter plate liquid scintillation counter (Top Count NXT)	Packard		Microplate Scintillation and Luminescence counter
Vial liquid scintillation counter (Beta-Couter 2500 TR)	Packard		Vial Scintillation and Luminescence counter
Liquid handler (Biomek 2000)	Beckman coulter		To prepare compound dilutions
Vortex (Vortex Genie 2)	Scientific industries Inc.		To mix compound stock solutions
Tissue homogenizer (Polytron PT1200E)	Kinematica AG		To resuspend the membrane preparations
XLfit5 software	Microsoft Excel add-on by IDBS		Curve fitting software
Smart Pull	UniPix	-	Electrode Puller
Roboject	MultiChannel Systems	-	Automated Injection (Xenopus Oocytes)
HiClamp	MultiChannel Systems	-	Automated Voltage Clamp (Xenopus Oocytes)
DataMining	MultiChannel Systems	-	Data Analysis Software
DataMerger	MultiChannel Systems	-	Data Processing Software
Digidata 1550	Molecular Devices		Low noise data acquisition system
CyberAmp 380 or equivalent	Axon Instruments		Programmable Signal Conditioner
pClamp program suite	Molecular Devices		Software for data acquisition and analysis software
Antivibraton table	TMC		To avoid microelectrode vibrations
Patchstar Micromanipulators	Scientifica		To accurately position microelectrodes in brain slices
Faraday Cage	Sutter Instruments		To isolate recordings from noise
Mcllwain Tissue Chopper	Campden Instruments LTD		To prepare brain slices
Borosilicate glass micropipette	Warner Instruments	GC150TF-10	To record extracellular potentials in brain slices
Twisted pair, platinum iridium wires stimulation electrode	World Precision Instruments		To stimulate the brain slices with current
Stimulus generator for current and voltage driven stimulation	MultiChannel Systems	STG4000	Delivers the current to the stimulation electrode
Plasticware			
Microtiter plate round bottom	Corning	#3365	Used for the binding assay

GF/C glassfibre filter-bottom 96-well microplate with 1.2 µm poresize, for cell harvesting assays using a vacuum manifoldwell filter	Packard	#6005174	used for the binding assay
50 mL Tubes	Falcon	#352070	used for the binding assay
Safe-lock tubes 1.5 mL	Eppendorf	#0030 120086	used for the binding assay
Safe-lock tubes 2 mL	Eppendorf	#0030 120094	used for the binding assay
Shipping tubes 180 mL	Semadeni Europe AG	#3722	used for the binding assay
Pony vial Polyethylene 6 mL	Perkin Elmer	#6013329	used for the binding assay
Top Seal	Perkin Elmer	#60051859	used for the binding assay
Spinner Flask	Bellco	BELLCO # 1965-00100	Spinner Flask
96 Well Plate (Conical)	Thermo Scientific Milian	56368	Injection plate for the oocytes
96 Well Plate (Flat bottom)	Corning (Vitaris Switzerland)	3364-cor	Compound Plate for the HiClamp
Glass capillary	Hilgenberg (Germany)	-	borosilicate glass 1.2 O.D. 0.76 I.D. with filament
RNA preparation			
Ambion mMessage mMachine	Thermo Fisher Scientific		for the <i>in vitro</i> synthesis
Chemicals			
Assay buffer: KCl 5 mM; CaCl ₂ 1.25 mM; MgCl ₂ 1.25 mM; NaCl 120 mM; Tris 15 mM pH adjust with HCl to 7.4; store up to 3 months at 4 °C.			Used for binding assay
Washing buffer: Tris 50 mM -HCl pH 7.4; store at 4 °C.			Used for binding assay
Stock solution of test compounds 10 mM in DMSO			Used for binding assay
Flumazenil (RO0151788) 10 mM in DMSO			Used for binding assay
Diazepam 4 mM in DMSO			Used for binding assay
[³ H]-Flumazenil 60-85 Ci/mmol in Ethanol; store at -20 °C			Used for binding assay
Microscint 40	Packard	#6013641	Used for binding assay
Ultima Gold	Perkin Elmer	#6013329	Used for binding assay
MS-222	Sigma	Sigma # A5040	MS-222
AB/AM	Sigma	Sigma # A5955	antibiotics / antimycotics
Collagenase	Sigma	Sigma # C1030	Collagenase Type I
Rnase	Sigma	Sigma # R2020-250 mL	RNaseZAP
EndoFree Plasmid maxi Kit	Qiagen	Qiagen # 12362	
Salts for artificial cerebrospinal fluid (NaCl, KCl, etc.)	Sigma		
Membranes			
Frozen membrane preparations from transient or stably transfected HEK293 overexpressing different human GABA _A receptor subtypes: α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2. See Ballard <i>et al.</i> 2009 for detailed methods.			Used for binding assay