

Materials List for:

Dissection of Local Ca²⁺ Signals in Cultured Cells by Membrane-targeted Ca²⁺ Indicators

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Materials

Name	Company	Catalog Number	Comments
(RS)-3,5-Dihydroxyphenylglycine (DHPG)	Tocris	#0342	
0.5% DNase I stock solution	Sigma-Aldrich	#11284932001	Prepare 0.5% DNase I (w/v) in Hanks' Balanced Salt Solution supplemented with 120 mM MgSO ₄ . Prepare 160 µL aliquots and store at -30 °C.
0.5% Trypsin-EDTA solution	Thermo Fisher Scientific	#25300054	
100 mM L-glutamine (×100 stock)	Thermo Fisher Scientific	#25030081	Preparing small aliquots of 250-750 µL and store at -30 °C.
100 mM Sodium pyruvate (×100 stock)	Thermo Fisher Scientific	#11360070	Aliquots (10 mL) can be stored at -20 °C. After thawing, the solution can be maintained at 4 °C for 2 months.
12-Well multiwell culture plates with low-evaporation lid	Falcon	#353043	Low-evaporation lid is critical for culturing neuron-glia mixed culture. For cell line cells, alternative culture dishes can be used.
18 mm diameter circular coverslips	Karl Hecht "Assistent"	#41001118	Thickness 1, 18 mm diameter circular coverslips; alternative coverslips can be used.
1 M HEPES	Thermo Fisher Scientific	#15630080	pH 7.2-7.6
2.5% Trypsin stock solution (×20 stock)	Sigma-Aldrich	#T4674	Prepare 150 µL aliquot and store at -30 °C.
50% Poly(ethyleneimine) (PEI) solution	Sigma-Aldrich	#P3143	Prepare 2% (V/V) PEI stock solution (×50) with distilled water sterilized by filtration. Store stock solution at -30 °C after preparing small aliquots of 250-750 µL. Prepare 0.04% PEI solution with distilled water on the day of coverslip coating.
70% Ethanol			Kept in a spray bottle to be used for surface disinfection.
Adeno-associated virus (AAV) for Lck-GCaMP6f, Lck-RCaMP2, and OER-RCaMP2 expression under the direction of the EF1a promoter			AAV can be prepared using AAV Helper Free System (Agilent Technologies) and HEK293 cells, or alternative methods. pAAV.EF1a.Lck-GCaMP6f, pAAV.EF1a.Lck-RCaMP2, and

			pAAV.EF1a.OER-GCaMP6f are available upon request.
B-27 supplement (×50 stock)	Thermo Fisher Scientific	#17504044	This can be replaced by B-27 plus supplement (Thermo Fisher Scientific; #A3582801) or MACS NeuroBrew-21 (Miltenyi Biotec, Bergisch Gladbach, Germany; #130-093-566).
B57BL/6	Japan SLC, Inc.		
Camera for microscopic image recording			The following cameras were available for use: cooled-CCD camera (e.g., Hamamatsu Photonics, OECA-ER), EM-CCD camera (e.g., Hamamatsu Photonics, ImagEM; Andor, iXon) or CMOS camera (e.g., Hamamatsu Photonics ORCA-Flash4.0)
Cell freezing container	Sarstedt K.K.	#95.64.253	Alternative cell freezing container can be used.
Cell strainer	Falcon	#352350	
CO ₂ incubator			Maintain at 37 °C, 5% CO ₂ .
Cryogenic tube	Corning	#430661	Alternative cryogenic tubes can be used.
Cryopreservation medium	Zenoaq		"CELLBANKER1"
Culture medium (for HeLa cells)			Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin solution (final concentration: Penicillin 100 U/mL and Streptomycin 100 µg/mL)
Dissection medium			One milliliter of 1 M HEPES (final concentration 20 mM) to 49 mL DMEM
DMEM	Nacalai	#08456-65	Alternative DMEM can be used.
DMEM	Nacalai	#08456-65	Low glucose
DNA transfection reagent	Sigma-Aldrich	#6366244001	"X-tremegene HP DNA transfection reagent" Alternative transfection reagents can be used.
Glass jar with a lid			500 mL jar (for mouse) or 1,500 mL jar (for rat) to anesthetize the animal
HBSS	Thermo Fisher Scientific	#14170161	HBSS free of calcium and magnesium
Heat inactivated bovine serum	Thermo Fisher Scientific	#10100147	
HeLa cells	RIKEN BioResource Center	#RCB0007	
Histamine	Sigma-Aldrich	#H7125	
Image analysis software			Such as Metamorph (Molecular Devices), ImageJ (NIH), and T1 Workbench14 (custom made)
Image splitting optics	Hamamatsu Photonics	#A12801-01	W-view GEMINI
Image splitting optics dichroic mirror	Semrock	#FF560-FDi01-25×36	For separation of green fluorescent protein/red fluorescent protein (GFP/RFP) signal
Image splitting optics emission filters	Semrock	#FF01-512/25-25, #FF01-630/92-25	For emission of GFP/RFP signal, respectively

Imaging medium and buffer			Use optimal medium or buffer for the experiment. When medium is used, medium without phenol red is desirable to reduce background fluorescence. Add 20 mM HEPES to maintain pH outside of CO ₂ incubator.
Incubation saline			Add 1 mL of 1 M HEPES (20 mM) to 49 mL HBSS
Inverted fluorescence microscope			Such as IX73 (Olympus) or Eclipse TI (Nikon Instech)
Isoflurane	Pfizer		Used for anesthesia
Maintenance medium (for 4 × 12 well dishes)			48.5 mL Neurobasal-A medium supplemented with 1 mL B-27, 500 μL of L-glutamine stock and 25 μL Penicillin-Streptomycin solution.
Maintenance medium for frozen cortical cells (for 1 × 12 well dishes)			12.2 mL Neurobasal plus medium supplemented with 250 μL B-27, 125 μL of L-glutamine stock and 6.2 μL Penicillin-Streptomycin solution.
MEM (Minimum Essential Medium)	Thermo Fisher Scientific	#11090-081	
Microscope filter set for GCaMP6f imaging			Appropriate filter for GFP (excitation, 480 ± 10 nm; emission, 530 ± 20 nm)
Microscope filter set for RCaMP2 imaging			Appropriate filter for RFP (excitation, 535 ± 50 nm; emission, 590 nm long pass)
Microscope filter sets for double imaging of RCaMP2 and GCaMP6f	Semrock	#FF01-468/553-25, #FF493/574-FDi01-25×36, #FF01-512/630-25	Dual excitation filter, Dual dichroic mirror, and emission filter for GFP/RFP imaging.
Microscope heating system			A heating system to maintain cells at 37 °C during the imaging. To avoid drift caused by thermal expansion, heating systems covering the entire microscope itself (e.g., Tokai Hit, Thermobox) are recommended.
Microscope light source for excitation			Mercury lamp (100 W), xenon lamp (75 W), Light-emitting diode (LED) illumination system (e.g., CoolLED Ltd., precisExcite; Thorlabs Inc., 4-Wavelength LED Source; Lumencor, SPECTRA X light engine). In case of mercury lamp and xenon lamp, use ND filter to reduce the excitation intensity.
Microscope objective lens			Plan-Apochromat oil immersion objective with numerical aperture higher than 1.3 is highly recommended for the recording of spontaneous Ca ²⁺ activity in neurons and astrocytes.
Neurobasal plus medium	Thermo Fisher Scientific	#A3582901	
Neurobasal-A Medium	Thermo Fisher Scientific	#10888022	Neurobasal plus medium (Thermo Fisher, A3582901) can be used instead of Neurobasal-A medium.
PBS(-): Phosphate-buffered saline free of Ca ²⁺ and Mg ²⁺	Fujifilm Wako Pure Chemical Cooperation	#164-23551	The absence of Ca ²⁺ and Mg ²⁺ is critical not to inhibit the trypsin activity. An alternative to PBS(-) can be used.

PC and image acquisition software			Such as Metamorph (Molecular Devices); Micromanager; TI Workbench ¹⁴ .
Penicillin-Streptomycin solution	Thermo Fisher Scientific	#15140122	Penicillin 10,000 U/mL and Streptomycin 10,000 µg/mL
Plasmid for Lck-GCaMP6f, Lck-RCaMP2, and OER-RCaMP2 expression under cytomegalovirus promoter ⁷⁻⁹			Available upon request
Plating medium (for 4 × 12 well dishes)			48 mL MEM supplemented with 1 mL B-27 supplement, 500 µL L-glutamine stock (final concentration: 2 mM), 500 µL of sodium pyruvate stock (1 mM) and 25 µL Penicillin-Streptomycin solution (penicillin 5 U/mL, streptomycin 5 µg/mL). This concentration of Penicillin-Streptomycin, which is 1/20 of the concentration recommended by the manufacturer, is critical for neuronal survival.
Recording chamber	Elveflow	Ludin Chamber	This recording chamber is for 18 mm diameter round coverslips.
Reduced serum media	Thermo Fisher	#11058021	Opti-MEM
Stereomicroscope			Used to dissect hippocampi. Olympus SZ60 or equivalent stereomicroscopes are available.
Surgical instruments			Standard dissecting scissors to cut the abdomen of a mouse or a rat, tweezers to pinch the uterus, delicate dissecting scissors to cut the uterus and the head of embryo, ring forceps to pinch the embryos, 13 cm curved Semken forceps (Fine Science Tools #11009-13) to extract brains, 3 forceps with fine tips (Dumont Inox #5)
Transfection reagent for neuron	Thermo Fisher Scientific	#L3000008	"Lipofectamine 3000" reagent. It is composed of the the "supplement (P3000)" that should be mixed with plasmid DNA in the step 2.2.3, and the "transfection reagent (lipofectamine 3000)" used in the step 2.2.4.
Trypan blue (0.4%)	Thermo Fisher Scientific	#15250061	
Wash medium for frozen cortical cells			25 mL DMEM, supplemented with 250 µL heat-inactivated fetal bovine serum + 12.5 µL Penicillin Streptomycin.
Wistar rats	Japan SLC, Inc		Pregnant rats (E18)