

Materials List for

Using Live Cell STED Imaging to Visualize Mitochondrial Inner Membrane Ultrastructure in Neuronal Cell Models

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Materials

Name	Company	Catalog Number	Comments
0.05% Trypsin-EDTA	Gibco	25300054	
0.4% Trypan blue	Invitrogen	T10282	
0.5% Trypsin-EDTA, no phenol red	Gibco	15400054	
100 X antibiotic-antimycotic	Gibco	15240062	
100 X/1.40 UPlanSApo oil immersion lens	Olympus		Equipped in Olympus IX83 microscope for STED setup described in Section 4
All-trans-retinoic acid	Sigma	R2625	
Amyloid- β (1-42, HiLyte Fluor647, 0.1 mg)	AnaSpec	AS-64161	Other fluorescent conjugates available
B27 supplement (50 X), serum free	Gibco	17504044	
Cell Counter (Countess II FL)	Life Technologies	AMQAF1000	
Centrifuge	Eppendorf	5804-R	
Counter slides	Invitrogen	C10283	
Conical tubes (15 mL)	Thermo Fisher Scientific	339650	
Cuvettes (Quartz Cells)	Starna Cells, Inc.	9-Q-10	Used with Spectrometer as described in Section 1.3
DMEM (high glucose with sodium pyruvate)	Gibco	11995073	Used for SH-SY5Y cell materials as described in Section 1
DMEM (high glucose no sodium pyruvate)	Gibco	11965092	Used for primary cell materials as described in Section 2
DMEM (phenol red-free)	Gibco	31053028	Used for imaging as described in Section 3
DMSO	Sigma	D8418	
DNAase I from bovine pancreas	Sigma	DN25	Used for primary cell materials as described in Section 2.2.1 and 2.2.2
DPBS (no calcium, no magnesium)	Gibco	14190144	
E18 Rat Hippocampus	Transnetyx Tissue	SDEHP	
Ethanol (200 proof)	Fisher Bioreagents	BP28184	
Fetal bovine serum (FBS), not heat-inactivated	Gibco	26140079	For cultured cells, in Section 1

Fetal bovine serum (heat inactivated)	Gibco	10082147	For primary cell culture, Section 2
Filter sterilization unit (0.1 µm, 500 mL)	Thermo Fisher Scientific	5660010	
FIJI (Is Just ImageJ) and Trainable Weka Segmentation (TWS) plug-in	--	--	Free, open-source image analysis software that includes plug-ins including Trainable Weka Segmentation described in Section 5; TWS plug-in from ref. 90 of the main text
GlutaMAX supplement (100 X)	Gibco	35050061	Glutamine supplement used for primary cell materials described in Section 2.1.2
Hausser Scientific bright-Line and Hy-Lite Counting Chambers	Hausser Scientific	267110	
HBSS (no calcium, no magnesium)	Gibco	14170120	Used for primary cell materials described in Section 2.2.1 and 2.2.2
HEPES	Gibco	15630080	
Huygens Professional deconvolution software (V. 20.10)	Scientific Volume Imaging (SVI)	--	The deconvolution software used in this protocol and described in Section 5
IX83 inverted microscope with Continuous Autofocus	Olympus	--	This paper uses a STED Infinity Line system built around an Olympus IX83 inverted microscope, described in Section 4
Lightbox software (V. 16.3.16118)	Abberior	--	Vendor software used for STED image acquisition, described in Section 4
Live Orange Mito dye	Abberior	LVORANGE-0146-30NMOL	Live cell imaging IMM-targeting dye described in Discussion
Neurobasal media	Gibco	21103049	Used for primary cell materials referred to in Section 2.1.2
Nunc Lab-Tek II 2-well chambered coverglass	Nunc	155379	Can purchase a variety of chambers but make sure the coverglass is #1.5
Pasteur Pipets (Fisherbrand)	Thermo Fisher Scientific	22183632	
Penicillin-Streptomycin (10,000 U/mL)	Gibco	15140122	
PKmito Orange dye	Spirochrome	SC053	
Poly-D-lysine	Gibco	A3890401	
SH-SY5Y Cell line	ATCC	CRL2266	
Sodium pyruvate (100 mM)	Gibco	11360070	Used for primary cell materials described in Section 2
Spectrometer (GENESYS 180 UV-Vis)	Thermo Fisher Scientific	840309000	
STED Expert Line microscope	Abberior	--	STED setup can be customized, but at time of purchase instrument was considered Abberior's Expert Line; brief description of setup is available in Section 4 of protocol
T25 flask (TC-treated, filter cap)	Thermo Fisher Scientific	156367	Other culture vessels and sizes available