

Materials List for:

Correlative Light Electron Microscopy (CLEM) for Tracking and Imaging Viral Protein Associated Structures in Cryo-immobilized Cells

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Materials

Name	Company	Catalog Number	Comments
UV Crosslinker	Stratagene	400072	Stratalinker 1800, 230 Vac, equipped with 254-nm UV light bulbs, 8 Watts/each.
Patterned sapphire discs	Engineering Office M. Wohlwend, Sennwald, Switzerland	627	They have a 0.3 mm diameter and are 0.16 mm thick, as well as an etched alphanumeric pattern to allow for re-location of the cells. They can be only ordered per e-mail: martin-wohlwend@bluewin.ch.
Slim and long tweezers	Electron Microscopy Sciences, Hatfield, PA, USA	72919-SS	"Style SS", for handling sapphire discs.
Cell culture medium	Thermo Fisher Scientific, Waltham, MA, USA	41965-062	Dulbecco's modified Eagle medium (DMEM) supplemented with 2 mM L-glutamine, nonessential amino acids, 100 units penicillin per ml, 100 µg streptomycin per ml and 10% fetal calf serum (DMEM complete, see below). Package containing 10 bottles, 500 mL/each.
L-glutamine	Thermo Fisher Scientific, Waltham, MA, USA	25030-123	Package containing 20 bottles of 100 mL/each.
Nonessential amino acids	Thermo Fisher Scientific, Waltham, MA, USA	11140-068	Package containing 20 bottles of 100 mL/each.
Penicillin/Streptomycin	Thermo Fisher Scientific, Waltham, MA, USA	15140-163	Package containing 20 bottles of 100 mL/each.
Fetal calf serum	Sigma Aldrich, St. Louis, MI, USA	F7524	Bottle of 50 mL
Inverted microscope	Olympus Deutschland, Hamburg, Germany	CKX41	It is an inverted microscope with trinocular head options and fluorescence upgrade capability.
Huh7-Lunet cells stably expressing the T7 RNA-polymerase	Available at Prof. Dr. Ralf Bartenschlager laboratory	Not available	Contact Prof. Bartenschlager at: ralf.bartenschlager@med.uni-heidelberg.de .
Mirus TransIT-LT1 Transfection Reagent	Mirus Bio LLC, Madison, USA	MIR 2304	Broad spectrum, low toxicity, DNA transfection reagent. Vial of 0.4 mL
Inverted widefield fluorescence microscope	Carl Zeiss Microscopy GmbH, Germany	Zeiss Observer.Z1	Inverted fluorescence microscope for experiments involving living cell cultures.
1-hexadecene	Merck, Darmstadt, Germany	8220640100	Bottle of 100 mL

"A" aluminium holders for high pressure freezing (HPF)	Engineering Office M. Wohlwend, Sennwald, Switzerland	241	This is the holder that has been used for this protocol and has 2 different depths of 0.1 and 0.2 mm. It can be only ordered per e-mail: martin-wohlwend@bluewin.ch.
"B" aluminium holders for HPF	Engineering Office M. Wohlwend, Sennwald, Switzerland	242	This is the holder that has been used for this protocol. It is 0.3 mm thick. They can be only ordered per e-mail: martin-wohlwend@bluewin.ch.
"A" aluminium holder for HPF (special for working with patterned sapphire discs)	Engineering Office M. Wohlwend, Sennwald, Switzerland	737	This is the holder than can be used alone with the patterned sapphire disc (as an alternative to the current protocol), without the need of a "B" holder or the edition of the "A" holder. It is 0.84 mm thick. It can be only ordered per e-mail: martin-wohlwend@bluewin.ch.
Sapphire discs	Engineering Office M. Wohlwend, Sennwald, Switzerland	405	These sapphires discs are the "conventional" one, with a 0.3 mm diameter and a thickness of 0.05 mm. They can be also used for CLEM as an alternative instead of patterned sapphire discs.
Finder grids	Electron Microscopy Sciences, Hatfield, Philadelphia, USA	LF135-Cu	These 135 mesh grids are used to create an alphanumeric pattern on "conventional" sapphire discs (described above) via carbon coating, so that they can be used for CLEM. 100 grids/vial.
HPF machine	ABRA Fluid AG, Widnau, Switzerland	HPM 010	This HPF machine has been used for this protocol.
HPF machine	Leica Microsystems, Vienna, Austria	EMPACT 2	"EMPACT 2", as an alternative to the use of the HPM machine that it has been used in this protocol (described above).
Cryo-tubes	Thermo Fisher Scientific, Waltham, MA, USA	Z763667-500EA	For long term-storage of cryo-immobilized samples in liquid nitrogen. Package containing 500 cryovials.
Liquid nitrogen dewar	Cole-Parmer GmbH, Wertheim, Germany	GZ-05094-60	For long term-storage of cryo-immobilized samples in liquid nitrogen, equipped with cryo-racks with a capacity of 1600 cryo-tubes.
Automatic freeze substitution (AFS) machine	Leica Microsystems, Vienna, Austria	EM AFS2	This machine performs freeze substitution and progressive lowering of temperature (PLT) techniques and allows low temperature embedding and polymerization of resins.
Osmium tetroxide (OsO ₄)	Electron Microscopy Sciences, Hatfield, PA, USA	19150	4% aqueous solution, one box containing 10 x 2 mL ampules.
Uranyl-Acetate (UA)	Serva, Heidelberg, Germany	77879.01	Bottle containing 25 grs of (UA)-2 H ₂ O.
Sonicator	Bandelin Electronic, Berlin, Germany	329	"Sonorex Super RK 31" is a high-power ultrasonic cleaning bath for aqueous cleaning solution that is used in this protocol to mix OsO ₄ and UA when preparing the FS medium.
Glass-distilled acetone	Electron Microscopy Sciences, Hatfield, PA, USA	10015	Bottle of 100 mL

Stereomicroscope	Leica Microsystems, Vienna, Austria	Leica M80	Routine stereomicroscope for daily inspections, equipped with a camera for capturing images.
Epoxy resin	Electron Microscopy Sciences, Hatfield, PA, USA	14120	Embed 812 is a kit containing several components that must be mix together in the proportions given by the manufacturers.
Flow through rings	Leica Microsystems, Vienna, Austria	16707157	Package containing 100 pieces.
Reagent baths	Leica Microsystems, Vienna, Austria	16707154	Package containing 100 pieces.
Ultramicrotome	Leica Microsystems, Vienna, Austria	Leica EM UC7	Ultramicrotome for preparation of semi- and ultrathin sections at room temperature.
Ultra 35 ° diamond knife	Diatome Ltd., Nidau Switzerland	AGG339-735	This knife have an edge length of 3 mm.
Ultra fine tweezers	Electron Microscopy Sciences, Hatfield, PA, USA	E78318	"Style 3X", for handling EM grids.
EM slot grids	Electron Microscopy Sciences, Hatfield, PA, USA	G2010-Cu	100 grids/vial.
EM grid storage box	Electron Microscopy Sciences, Hatfield, PA, USA	71155	It has capacity for 100 grids.