

# Rapid Freezing using Sandwich Freezing Device for Good Ultrastructural Preservation of Biological Specimens in Electron Microscopy

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## Materials

Name	Company	Catalog Number	Comments
Sandwich Freezing Device	Marine works Japan, Ltd, Yokosuka, Japan	MW-SFD-01	with metal bar, thin metal bar, tweezers, and working bath
10 mL glass vials		-	Scintillation counter vials for fixative
Acetone		-	
Osmium tetroxide	Nisshin EM Co. Ltd., Tokyo	3004	0.1 g
Deep freezer	Sanyo Co. Ltd., Osaka	MDF-C8V1	
Copper disk	Nisshin EM Co. Ltd., Tokyo	-	Refer to this paper
Slide glass		-	
Double-sided adhesive tape		-	
Single-edged razor blade	Nisshin EM Co. Ltd., Tokyo	-	Feather, FAS-10
Double-edged razor blade	Nisshin EM Co. Ltd., Tokyo	-	Feather, FA-10
Shredded board	Nisshin EM Co. Ltd., Tokyo	428	
Tweezers	Nisshin EM Co. Ltd., Tokyo	-	Several pairs
Tweezers with polystyrene foam		-	One pair
Glutaraldehyde	Nisshin EM Co. Ltd., Tokyo	3052	
Liquid nitrogen		-	
Propane gas		-	Cryogen
Ion sputter apparatus	Hitachi high technologies, Tokyo	Hitachi E102	
Micropipette		-	For 1 mL, 200 $\mu$ L, and 2 $\mu$ L
Microcentrifuge	Tomy digital biology Co. Ltd., Tokyo	Capsulefuge, PMC-060	
Stereomicroscope	Nikon Co. Ltd., Tokyo et al.	-	SMZ 645
LED illumination for stereomicroscope	Nikon Co. Ltd., Tokyo et al.	SM-LW 61 Ji	
Disposable plastic container		-	50 mL and 200 mL

Ethane gas		-	Cryogen
2 mL Eppendorf tubes		-	For embedding
Disposable plastic syringes		-	1 mL, 5 mL, 10 mL, and 20 mL
Magnetic stirrer		-	
Epoxy resin	Nisshin EM Co. Ltd., Tokyo	340	Quetol 812 set
Silicon embedding mold	Nisshin EM Co. Ltd., Tokyo	4217	7 mm in diameter, 13 mm deep
Incubater		-	For 37 °C and 60 °C
Trimming stage	Sunmag Co. Ltd., Tokyo	-	Tilting mechanism equipped, Refer to this paper
LED illumination for trimming stage	Sunmag Co. Ltd., Tokyo	-	Refer to this paper
Ultrasonic Trimming Blade	Nisshin EM Co. Ltd., Tokyo	5240	EM-240, Refer to this paper
Ultramicrotome	Leica Microsystems, Vienna	Ultracut S	
Grids	Nisshin EM Co. Ltd., Tokyo	2633, 2634	300 mesh, 400 mesh
0.5% Neoprene W solution	Nisshin EM Co. Ltd., Tokyo	605	
Perfect Loop	Nisshin EM Co. Ltd., Tokyo	2351	Fot retrieving sections
Half Tube for section staining	Nisshin EM Co. Ltd., Tokyo	463	Refer to this pape
Super Support Film	Nisshin EM Co. Ltd, Tokyo	647	
Syringe filter	Toyo Roshi Kaisha, Ltd., Tokyo	DISMIC-03CP	Cellulose acetate, 0.45 µm
Transmission electron microscope	JEOL Co. Ltd., Tokyo	JEM-1400	