

Materials List for:

Inducing Ischemia-reperfusion Injury in the Mouse Ear Skin for Intravital Multiphoton Imaging of Immune Responses

Chi Ching Goh^{1,2}, Jackson Liang Yao Li¹, David Becker³, Wolfgang Weninger^{4,5,6}, Veronique Angeli^{2,7}, Lai Guan Ng^{1,2,5,8}

¹Singapore Immunology Network (SigN), Agency for Science, Technology and Research (A*STAR), Biopolis

²Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore

³Lee Kong Chian School of Medicine, Nanyang Technological University

⁴Centenary Institute for Cancer Medicine and Cell Biology

⁵Discipline of Dermatology, University of Sydney

⁶Department of Dermatology, Royal Prince Alfred Hospital

⁷LSI Immunology Programme, National University of Singapore

⁸School of Biological Sciences, Nanyang Technological University

Correspondence to: Lai Guan Ng at Ng_Lai_Guan@immunol.a-star.edu.sg

URL: <https://www.jove.com/video/54956>

DOI: [doi:10.3791/54956](https://doi.org/10.3791/54956)

Materials

Name	Company	Catalog Number	Comments
Mice strains			
Lysozyme-GFP C57BL/6	Thomas Graf, Center for Genomic Regulation		
C57BL/6-C2J	Jackson Laboratories	000058	To be crossed with Lysozyme-GFP to generate albino Lysozyme-GFP for skin imaging
Reagents			
PBS			
Viaflex 0.9% (wt/vol) saline	Baxter Healthcare	F8B1323	
Ketamine (100 mg mL ⁻¹ ketamine hydrochloride)	Parnell		Ketamine is a controlled drug and all relevant local regulations should be followed
Ilium Xylazil-20 (20 mg mL ⁻¹ xylazine hydrochloride)	Troy Laboratories		Xylazil-20 is a controlled drug and all relevant local regulations should be followed.
Evans blue (10 mg mL ⁻¹ in PBS or saline)	Sigma-Aldrich	46160	
Ultrapurified water			
Equipment			
Insulin syringe with needle	BD	328838	
Transfer pipettes	Biologix Research Company	30-0135	
3 M paper masking tape	3M	2214	
Deckglaser microscope cover glass (22 mm × 32 mm)	Paul Marienfeld	101112	
Curved splinter forceps	Aesculap, B. Braun Melsungen	BD312R	
Veet hair removal cream	Reckitt Benckiser		
Medical cotton-tipped applicators	Puritan Medical Products Company	806-WC	
C-fold towels	Kimberly-Clark	20311	
Kimwipes delicate task wipes	Kimtech Science	34155	
Gold-plated, N42-grade neodymium magnets, 12 mm in diameter and 2 mm thick	first4magnets	F656S	

Plastic guide, 10 cm by 1.5 cm (polyvinyl chloride material)			fold in half lengthwise, bind with masking tape and slot magnet in
High vacuum grease	Dow Corning		
Microscope			
TriM Scope II single-beam two-photon microscope	LaVision BioTec		
Tunable (680–1,080 nm) Coherent Chameleon Ultra II One Box Ti:sapphire laser (≥3.3 W at 800 nm; pulse length of 140 fs, 80 MHz repetition rate)	Coherent		
Water-dipping objectives (20×, NA = 1.0)	Olympus	XLUMPLFLN20xW	
Microscope filter and mirror sets (for imaging GFP, SHG, Evans Blue)			
495 long-pass	Chroma	T495LPXR	
560 long-pass	Chroma	T560LPXR	
475/42 band-pass	Semrock	FF01-475/42-25	
525/50 band-pass	Chroma	ET525/50m	
655/40 band-pass	Chroma	NC028647	
Skin-imaging stage platform (refer to diagram for assembly)			
A metal base plate (126 mm × 126 mm × 1 mm)			
A brass platform for the ear (79 mm × 19 mm; 1 mm thickness at side, 0.5 mm thickness in the middle; Figure 1) with slit (1.7 mm × 1 mm; 1.5 mm away from long edge)			
Two plastic blocks (10 mm in height)—for heat insulation			
Curved holder, for positioning the control thermistor on the ear platform			
Interface cable CC-28 with DIN connector and thermistors, one for the temperature control and the other for the temperature monitor	(Warner Instruments (Harvard Apparatus))	640106	connect the interface cable to both resistive heater blocks set at 35 °C
Resistive heater blocks RH-2	(Warner Instruments (Harvard Apparatus))	640274	Resistive heater blocks can heat the brass ear platform up to over 100 °C within minutes. Ensure that the control thermistor has been properly secured in the holder in order to avoid overheating.
Temperature controller TC-344B for the ear platform	(Warner Instruments (Harvard Apparatus))	640101	
Temperature controller TR-200 for mouse heating pad	Fine Science Tools	21052-00	Unit is no longer for sale. Ask manufacturer for alternatives
Power supply for TR-200	Fine Science Tools	21051-00	Unit is no longer for sale. Ask manufacturer for alternatives
Heating pad	Fine Science Tools	21060-00	Unit is no longer for sale. Ask manufacturer for alternatives.
Animal rectal probe	Fine Science Tools	21060-01	Unit is no longer for sale. Ask manufacturer for alternatives. After connecting the rectal probe and heating pad to the temperature controller TR-200, set the temperature to 37 °C

Coverslip holder			
2 plastic rods, 1 cm in diameter, 10 cm in length			
1 plastic adaptor with holes drilled to accommodate rods (refer to diagram)			
3 plastic tightening screws for keeping plastic rods in place			
1 metal plate, 6 cm x 2.5 cm, with a 2 cm square cut at 1 end, 2 mm edge away from short edge			
1 pair of nut and bolt for attaching metal plate to plastic rod			
1 acrylic base (4 cm x 5 cm x 1.5 cm) with magnet to hold coverslip holder on skin-imaging stage platform. 1 rod is permanently fixed onto base.			
Imaging analysis software			
Imaris v8.1.2	Bitplane		