

Materials List for:

# High-Resolution 3D Imaging of Rabies Virus Infection in Solvent-Cleared Brain Tissue

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## Materials

Name	Company	Catalog Number	Comments
<b>Reagents</b>			
Benzyl alcohol	Alfa Aesar	41218	Clearing reagent
Benzyl benzoate	Sigma-Aldrich	BB6630-500ML	Clearing reagent
Dimethyl sulfoxide	Carl Roth	4720.2	Various buffers
Diphenyl ether	Sigma-Aldrich	240834-100G	Clearing reagent
DL- $\alpha$ -Tocopherol	Alfa Aesar	A17039	Antioxidant
Donkey serum	Bio-Rad	C06SBZ	Blocking reagent
Glycine	Carl Roth	3908.2	Background reduction
Goat serum	Merck	S26-100ML	Blocking reagent
Heparin sodium salt	Carl Roth	7692.1	Background reduction
Hydrogen peroxide solution (30 %)	Carl Roth	8070.2	Sample bleaching
Methanol	Carl Roth	4627.4	Sample pretreatment
Paraformaldehyde	Carl Roth	0335.3	Crystalline powder to make fixative solution
Sodium azide	Carl Roth	K305.1	Prevention of microbial growth in stock solutions
tert-Butanol	Alfa Aesar	33278	Sample dehydration for tissue clearing
TO-PRO-3	Thermo Fisher	T3605	Nucleic acid stain
Triton X-100	Carl Roth	3051.2	Detergent
Tween 20	AppliChem	A4974,0500	Detergent
<b>Miscellaneous</b>			
5 mL reaction tubes	Eppendorf	0030119401	Sample tubes
Coverslip, circular (diameter: 22 mm)	Marienfeld	0111620	Part of imaging chamber
Coverslip, circular (diameter: 30 mm)	Marienfeld	0111700	Part of imaging chamber
Hypodermic needle (27 G x 3/4" [0.40 mm x 20 mm])	B. Braun	4657705	Filling of the imaging chamber with clearing solution
RTV-1 silicone rubber	Wacker	Elastosil E43	Adhesive for the assembly of the imaging chamber
Ultimaker CPE 2.85 mm transparent	Ultimaker	8718836374869	Copolyester filament for 3D printer to print parts of the imaging chamber
<b>Technical equipment and software</b>			
3D printer	Ultimaker	Ultimaker 2+	Printing of imaging chamber

Automated water immersion system	Leica	15640019	Software-controlled water pump
Benchtop orbital shaker	Elmi	DOS-20M	Sample incubation at room temperature (~ 150 rpm)
Benchtop orbital shaker, heated	New Brunswick Scientific	G24 Environmental Shaker	Sample incubation at 37 °C (~ 150 rpm)
Confocal laser scanning microscope	Leica	DMI 6000 TCS SP5	Inverted confocal microscope for sample imaging
Fiji	NIH (ImageJ)	open source software (v1.52h)	Image processing package based on ImageJ
Long working distance water immersion objective	Leica	15506360	HC PL APO 40x/1.10 W motCORR CS2
Vibratome	Leica	VT1200S	Sample slicing
Workstation	Dell	Precision 7920	CPU: Intel Xeon Gold 5118 GPU: Nvidia Quadro P5000 RAM: 128 GB 2666 MHz DDR4 SSD: 2 TB
<b>Primary antibodies</b>			
Goat anti-RABV N	Friedrich-Loeffler-Institut		Monospecific polyclonal goat anti-RABV N serum, generated by goat immunization with baculovirus-expressed and His-tag-purified RABV nucleoprotein N Dilution: 1:400
Rabbit anti-GFAP	Dako	Z0334	Polyclonal antibody (RRID:AB_10013382) Dilution: 1:100
Rabbit anti-MAP2	Abcam	ab32454	Polyclonal antibody (RRID:AB_776174) Dilution: 1:250
Rabbit anti-RABV P 160-5	Friedrich-Loeffler-Institut		Monospecific polyclonal rabbit anti-RABV P serum, generated by rabbit immunization with baculovirus-expressed and His-tag-purified RABV phosphoprotein P (see reference 23: Orbanz et al., 2010) Dilution: 1:1,000
<b>Secondary antibodies</b>			
Donkey anti-goat IgG	Thermo Fisher Scientific	depending on conjugated fluorophore	Highly cross-absorbed Dilution: 1:500
Donkey anti-mouse IgG	Thermo Fisher Scientific	depending on conjugated fluorophore	Highly cross-absorbed Dilution: 1:500
Donkey anti-rabbit IgG	Thermo Fisher Scientific	depending on conjugated fluorophore	Highly cross-absorbed Dilution: 1:500
Goat anti-mouse IgG	Thermo Fisher Scientific	depending on conjugated fluorophore	Highly cross-absorbed Dilution: 1:500
Goat anti-rabbit IgG	Thermo Fisher Scientific	depending on conjugated fluorophore	Highly cross-absorbed Dilution: 1:500