

Materials List for:

Reliable Isolation of Central Nervous System Microvessels Across Five Vertebrate Groups

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Materials

Name	Company	Catalog Number	Comments
10X PBS	ThermoFisher	BP39920	Used for blocking and antibody diluent.
20% PFA	Electron Microscopy Sciences	15713-S	Used as fixative (4% PFA)
70,000 MW Dextran	Millipore Sigma	9004-54-0	Used for MV-2 solution
Adson Forceps	Fine Science Tools (FST)	11006-12	Used for removal of muscle and skin
Adson Forceps, student quality	FST	91106-12	Same as above but cheaper
Bovine serum albumin (BSA)	Millipore Sigma	A7906-100G	Used for MV-3 solution, blocking and antibody diluent
Corning 100 µm Cell strainer	Millipore Sigma	CLS431752-50EA	
Corning 70 µm Cell strainer	Millipore Sigma	CLS431751-50EA	
Corning Deskwork low-binding tips	Millipore Sigma	CLS4151	Same as below but cheaper.
Cultrex Poly-D-Lysine	R&D	3439-100-01	Used for slide coating
Donkey anti-Goat IgG-ALEXA 555	Thermo	A21432	Used as secondary antibody. Recommended dilution of 1:200.
Donkey anti-Mouse IgG-ALEXA 488	Thermo	A21202	Used as secondary antibody. Recommended dilution of 1:200.
Donkey anti-Rabbit IgG-ALEXA 488	Thermo	A21206	Used as secondary antibody. Recommended dilution of 1:200.
Donkey anti-Rabbit IgG-ALEXA 647	Thermo	A31573	Used as secondary antibody. Recommended dilution of 1:200.
Donkey anti-Rat IgG-DyLight 650	Thermo	SA5-10029	Used as secondary antibody. Recommended dilution of 1:200.
Double-Pronged Tissue Pick	FST	18067-11	Used for removal of meninges and choroid plexus
Dumont #3c Forceps	FST	11231-20	Used for more delicate and/or small CNS tissue handling (like pituitary)
Dumont #7 Forceps	FST	11274-20	Used for CNS tissue dissection and handling
Dumont #7 Forceps, student	FST	91197-00	Same as above but cheaper
ep Dualfilter T.I.P.S. LoRetention Tips	Eppendorf	22493008	Better quality than the tips above (more expensive).
Extra Fine Graefe Forceps, serrated	FST	11151-10	Used for bone removal
Fine Scissors, sharp	FST	14060-09	Used for removal of pig and macaque dural sac

Glass Pestle 1.5 mL Microcentrifuge Tube Tissue Grinder Homogenizer, Pack of 10	Chang Bioscience Inc. (eBay)	GP1.5_10	Used for small vertebrate hypothalamus and pituitary.
Goat anti-CXCL12, biotinylated	PeptoTech	500-P87BGBT	Used as primary antibody on CNS microvessels from all specimens. Recommended dilution: 1:20.
Goat anti-JAM-B	R&D	AF1074	Used as primary antibody to assess neuroinflammation. Recommended concentration: 5 µg/mL.
Goat anti-Mouse IgG-ALEXA 488	Thermo	A11001	Used as secondary antibody. Recommended dilution of 1:200.
Goat anti-Mouse IgG-ALEXA 555	Thermo	A21424	Used as secondary antibody. Recommended dilution of 1:200.
Goat anti-PDGFRβ	R&D	AF1042	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Goat anti-Rabbit IgG-ALEXA 555	Thermo	A21249	Used as secondary antibody. Recommended dilution of 1:200.
Goat anti-Rabbit IgG-DyLight 488	Thermo	35552	Used as secondary antibody. Recommended dilution of 1:200.
Goat anti-Rat IgG-DyLight 650	Thermo	SA5-10021	Used as secondary antibody. Recommended dilution of 1:200.
Graefe Forceps, curved tip, 1X2 teeth	FST	11054-10	Use for nylon filter net holding and shaking
HBSS, 1X buffer with calcium and magnesium	Corning	21-022-CM	Used for MV-1 solution
HEPES, 1M liquid buffer	Corning	25-060-CI	Used for MV-1 solution
Isolectin GS-IB4-Biotin-XX	ThermoFisher Scientific (Thermo)	I21414	Glycoprotein isolated from legume <i>Griffonia simplicifolia</i> that binds D- galactosyl residues of endothelial cell glycocalyx. Used for avian and porcine CNS microvessels. Recommended concentration: 5 µg/mL.
LaGrange Scissors, serrated	FST	14173-12	Used for skull dissection and laminectomy (except pig and macaque)
Millicell EZ slide 8-well unit	Millipore Sigma	PEZGS0816	
Mouse anti-CLDN5	Thermo	35-2500	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Mouse anti-GGT1	Abcam	ab55138	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Mouse anti-Human CD31	R&D	BBA7	Used as primary antibody on primate CNS microvessels. Recommended concentration: 16.5 µg/mL.
Mouse anti-NFM	Thermo	RMO-270	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Mouse anti-αSMA	Thermo	MA5-11547	Used as primary antibody on CNS microvessels from all specimens. Recommended dilution of 1:200.

Nylon Filter Net, roll	Millipore Sigma	NY6000010	Laser-cut to 13 mm diameter filter net discs. Used for small vertebrate hypothalamus and pituitary.
Nylon Filter Nets, 25 mm	Millipore Sigma	NY2002500	Used on most small vertebrates CNS tissues, except hypothalamus and pituitary. Used for macaque and pig hypothalamus and pituitary.
Nylon Filter Nets, 47 mm	Millipore Sigma	NY2004700	Used for macaque and pig CNS tissues, except hypothalamus and pituitary.
ProLong Gold antifade reagent with DAPI	ThermoFisher	P36935	Used to coverslip slides.
Rabbit anti-AQP4	Millipore Sigma	A5971	Used as primary antibody on CNS microvessels from all specimens. Recommended dilution of 1:200.
Rabbit anti-LSR	Millipore Sigma	SAB2107967	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Rabbit anti-NG2	Millipore Sigma	AB5320	Used as primary antibody on CNS microvessels from all specimens. Recommended dilution of 1:200.
Rabbit anti-OSP	Abcam	ab53041	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 1 µg/mL.
Rabbit anti-VE-Cadherin	Abcam	ab33168	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Rabbit anti-ZO-1	Thermo	61-7300	Used as primary antibody on CNS microvessels from all specimens. Recommended concentration: 5 µg/mL.
Rat anti-CD31	Becton Dickinson	BD 550274	Used as primary antibody for murine CNS microvessels. Recommended concentration: 5 µg/mL.
Rat anti-GFAP	Thermo	13-0300	Used as primary antibody on CNS microvessels from all specimens. Recommended dilution of 1:200.
Rat anti-VCAM-1	Becton Dickinson	BD 553329	Used as primary antibody to assess neuroinflammation. Recommended concentration: 5 µg/mL.
Sterile Ringer's Solution, Frog	Aldon Corporation	IS5066	Used for amphibian anesthesia
Streptavidin-ALEXA 555	Thermo	S32355	Used as secondary antibody to label biotinylated primary antibodies. Recommended dilution of 1:500.
Streptavidin-ALEXA 647	Thermo	S32357	Used as secondary antibody to label biotinylated primary antibodies. Recommended dilution of 1:500.
Surgical Scissors, sharp	FST	14002-12	Used for removal of muscle and skin
Surgical Scissors, sharp-blunt	FST	14001-16	Used for decapitation (except pig and macaque)

Swinnex Filter Holder, 13 mm	Millipore Sigma	SX0001300	Modified by laser-cut. Used for small vertebrate hypothalamus and pituitary.
Swinnex Filter Holder, 25 mm	Millipore Sigma	SX0002500	Modified by laser-cut. Used on most small vertebrates CNS tissues, except hypothalamus and pituitary. Used for macaque and pig hypothalamus and pituitary.
Swinnex Filter Holder, 47 mm	Millipore Sigma	SX0004700	Modified by laser-cut. Used for macaque and pig CNS tissues, except hypothalamus and pituitary.
Triton X-100	ThermoFisher	50-165-7277	Used for blocking and antibody diluent.
Wheaton 120 Vac Overhead Stirrer	VWR (Supplier DWK Life Sciences)	62400-904 (DWK #903475)	Used for macaque and pig CNS tissues with 55 mL tissue grinder, except hypothalamus and pituitary.
Wheaton Potter-Elvehjem tissue grinder with PTFE pestle, 10 mL	VWR (Supplier DWK Life Sciences)	14231-384 (DWK #357979)	Used on most small vertebrates CNS tissues, except hypothalamus and pituitary. Used for macaque and pig hypothalamus and pituitary.
Wheaton Potter-Elvehjem tissue grinder with PTFE pestle, 55 mL	VWR (Supplier DWK Life Sciences)	14231-372 (DWK #357994)	Used for macaque and pig CNS tissues, except hypothalamus and pituitary.