

Materials List for:

Production, Purification, and Quality Control for Adeno-associated Virus-based Vectors

Shelly Fripont^{*1,2}, Catherine Marneffe^{*1,2}, Marika Marino^{*1,2}, Melvin Y. Rincon^{1,2}, Matthew G. Holt^{1,2,3}

¹Flemish Institute for Biotechnology-Catholic University (VIB-KU) Leuven Center for Brain and Disease Research

²Department of Neuroscience, KU Leuven

³Leuven Brain Institute

*These authors contributed equally

Correspondence to: Melvin Y. Rincon at rinconmy@gmail.com, Matthew G. Holt at matthew.holt@kuleuven.vib.be

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Materials

Name	Company	Catalog Number	Comments
Plasmid production			
pTransgene plasmid	De novo design or obtained from a plasmid repository	N/A	See step 1 of main protocol for further details
pCapsid	De novo design or obtained from a plasmid repository	N/A	See step 1 of main protocol for further details
pHelper	Agilent	240071	
Plasmid <i>Plus</i> maxi kit	Qiagen	12963	
QIAquick PCR purification Kit	Qiagen	28104	
AAV Helper-Free System	Agilent	240071	
Cell culture and transfection			
Dulbecco's Modified Eagle Medium (DMEM), high glucose, no glutamine	Life technologies	11960-044	Supplement DMEM with FBS (1% or 10% v/v) and GlutaMAX 200 mM (1% v/v) then filter sterilize the medium using a 0.22 mm filter
Fetal bovine serum (FBS)	GIBCO	10500-064	
GlutaMAX supplement (200 mM)	GIBCO	35050038	
Corning bottle-top vacuum filter system	Sigma-Aldrich	CLS430769	
Dulbecco's Phosphate Buffered Saline (DPBS) with no calcium and no magnesium	GIBCO	14190094	
HEK293T cells	American Tissue Culture Collection	CRL3216	Upon receipt, thaw the cells and culture as described in the protocol. After a minimal number of passages, freeze a subfraction for future in aliquots. Always use cells below passage number 20. Once cultured cells have been passaged more than 20 times, restart a culture from the stored aliquots
Cell culture dishes	Greiner Cellstar	639160	15 cm diameter culture dishes
Cell scrapers	VWR	10062-904	
Polyethylenimine (PEI)	Polyscience	23966-2	PEI in powder form is dissolved at 1 µg/µL in deionized water (ddwater) at pH=2 (use HCl). Prepare in a beaker and stir for 2-3 h. When dissolved, bring the pH

			back to 7 with NaOH. Filter sterilize and store the resuspended stock solution in 1 ml aliquots at -20 °C. PEI aliquots can freeze/thawed multiple times.
Virkon solution	Fisher Scientific	NC9821357	Disinfect any material that has been in contact with assembled viral particles with Virkon solution
Mutexi long-sleeve aprons	Fisher Scientific	11735423	Wear an apron over the top of a regular lab coat
Fisherbrand maximum protection disposable overshoes	Fisher Scientific	15401952	
AAV Purification and desalting			
Optiprep density gradient medium	Sigma-Aldrich	D1556	Optiprep is a 60% (w/v) solution of iodixanol in water (sterile). CAUTION. Use under a laminar flow hood. Wear gloves
Phenol red	Sigma-Aldrich	P0290	CAUTION. Use under a laminar flow hood
Pasteur pipette	Sigma-Aldrich	Z627992	Sterilize before use
OptiSeal Polypropylene tubes	Beckman	361625	
Benzonase (250 U/ μ L)	Sigma-Aldrich	E1014	Supplied as a ready-to-use solution
Acrodisc syringe filter	Pall corporation	4614	
Omnifix syringe (5mL)	Braun	4617053V	
Blunt syringe needle	Sigma Aldrich	Z261378	Stainless steel 316 syringe needle, pipetting blunt 90° tip gauge 16, L 4 in. Referred to in the text as a blunt-end needle
Aqua Ecotainer	B. Braun	0082479E	Sterile endotoxin-free water. Referred to as 'Ultrapure water'
Amicon ultra-15 centrifugal filter unit	Millipore	UFC910024	These filters concentrate the final product by collecting the viral particles in consecutive centrifugation steps
Pluronic F68 (100X)	Thermo Fisher	24040032	Non-ionic surfactant. Dilute in sterile PBS to use at 0,01% (v/v)
Fisherbrand Sterile Microcentrifuge Tubes with Screw Caps (2 mL)	Fisher Scientific	02-681-374	Use skirted tubes for easy handling
AAV Titration			
Restriction enzyme: StuI (10 U/ μ L)	Promega	R6421	
DNAse I (1 U/ μ L)	Fisher scientific	EN0521	
Proteinase K	Sigma-Aldrich	3115852001	Reconstitute in ultrapure water and use at a final concentration of 10 mg/ml. Solution can be stored at -20°C
EasyStrip Plus Tube Strips (with attached caps)	Fisher scientific	AB2000	
Eppendorf microtube 3810x	Sigma-Aldrich	Z606340-1000EA	
LightCycler 480 SYBR Green I Master Mix	Roche	4707516001	
LightCycler Multiwell Plates, 96 wells	Roche	4729692001	White polypropylene plate (with unique identifying barcode)
Microseal 'A' PCR Plate and PCR Tube Sealing Film	Bio-Rad	msa5001	
AAV Purity control			

Ammonium persulfate (APS)	Sigma-Aldrich	A3678	Reconstitute in ultrapure water to 10% (v/v). CAUTION. Use under laminar flow hood. Wear gloves
Tetramethylethylenediamine (TEMED)	Sigma-Aldrich	T9281	CAUTION. Use under a laminar flow hood. Wear gloves
Tris Base ULTROL Grade	Merck	648311	CAUTION. Use under a laminar flow hood. Wear gloves
UltraPure Agarose	Thermo Fisher	16500-500	
Rotiphorese® Gel 30 (37,5:1)	Carl Roth	3029.3	Aqueous 30 % acrylamide and bisacrylamide stock solution at a ratio of 37.5:1. CAUTION. Use under laminar flow hood. Wear gloves
Serva Blue G	Sigma-Aldrich	6104-58-1	
Precision Plus prestained marker	Bio-Rad	1610374edu	
1-Butanol	Sigma-Aldrich	B7906	CAUTION. Use under a laminar flow hood. Wear gloves
Immunohistochemistry			
Rabbit anti-GFP	Synaptic System	132002	1:300 dilution
Anti-rabbit Alexa Fluor 488	Invitrogen	A21206	1:1000 dilution
Equipment	Company	Catalog number	Comments
Vector production lab			
Rotina 380 bench-top centrifuge *	Hettich	1701	
Optima XPN 80 ultracentrifuge *	Beckmann Coulter	A95765	
Type 50.2 Ti fixed-angle titanium rotor *	Beckmann Coulter	337901	
Entris digital scale *	Sartorius	2202-1S	
Warm water bath *			Set at 37°C
Ice bucket *	VWR	10146-290	Keep material used in the vector production lab separate from that used in standard lab areas
Pipetboy pro *	Integra	156,400	
Graduated pipettes: Cell star *	Greiner bio-one	606180	Capacity of 5 ml, 10 ml and 25 ml
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Graduated pipettes: Cell star *	Greiner bio-one	760180	Capacity of 5 ml, 10 ml and 25 ml
Co ₂ incubator CB150 *	Binder	9040-0038	Set at 37°C, 5% CO ₂ and 95% humidity
Nuaire safety cabinet NU 437-400E *	Labexchange	31324	Clean all the surfaces with 70% ethanol and Virkon before and after use
Conventional lab			
T100 thermal cycler *	Bio-Rad	1861096	
LightCycler 480 Instrument II *	Roche	5015278001	
ThermoMixer *	Eppendorf	C 5382000015	
Nanodrop *	ThermoFisher Scientific	ND 2000	
Mini-Protean Tetra Cell*	Bio-Rad	1658001FC	For use with handmade or precast gels
ProteoSilver silver stain kit	Sigma-Aldrich	PROTSIL1	High sensitivity protein detection with low background
Centrifuge 5804 R *	Eppendorf	B1_022628045	High speed centrifuge for medium capacity needs (up to 250 ml)
Graduated pipettes Cell star *	Greiner bio-one	606180	5 ml, 10 ml and 25 ml

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Graduated pipettes Cell star *	Greiner bio-one	760180	5 ml, 10 ml and 25 ml
Filter tips *	Greiner bio-one	750257	2 µl, 20 µl, 200 µl
Filter tips *	Greiner bio-one	738257	2 µl, 20 µl, 200 µl
Filter tips *	Greiner bio-one	771257	2 µl, 20 µl, 200 µl
Ice bucket with lid *	VWR	10146-290	
Mini diaphragm vacuum pump, VP 86 *	VWR	181-0065	
Pipetman P2, P20, P100, P200, P1000	Gilson	F144801	
Pipetman P2, P20, P100, P200, P1000	Gilson	F123600	
Pipetman P2, P20, P100, P200, P1000	Gilson	F123615	
Pipetman P2, P20, P100, P200, P1000	Gilson	F123601	
Pipetman P2, P20, P100, P200, P1000	Gilson	F123602	
* Materials marked with an asterisk are expensive pieces of equipment and are usually central infrastructure items shared between multiple labs. These items can also be replaced by equivalents if available. Note, when a different ultracentrifuge is used, care must be taken to select the correct rotor and centrifuge tubes.			