

Suspension Culture Production and Purification of Adeno-Associated Virus by Iodixanol Density Gradient Centrifugation for *In Vivo* Applications

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Materials

Name	Company	Catalog Number	Comments
5810 R benchtop centrifuge	Eppendorf	22625501	
8-channel peristaltic pump	Watson-Marlow	020.3708.00A	
Automated cell counter	NanoEntek	EVE-MC	
Avanti J-E high-speed centrifuge	Beckman Coulter	369001	
Benzonase	Thermo Scientific	88701	
Biological safety cabinet	Labconco	322491101	
CO ₂ incubator with shaker			Set at 8% CO ₂ and 37 °C
Conical centrifuge tubes	Thermo Scientific	339652	50 mL
Conical centrifuge tubes	Thermo Scientific	339650	15 mL
Disposable micro-pipets	Fisherbrand	21-164-2G	Capillaries
Dulbecco's phosphate buffered saline without CaCl ₂ and MgCl ₂ (DPBS) (10x)	Sigma-Aldrich	D1408	
ECLIPSE Ts2R-FL inverted microscope	Nikon		
Expi293 Expression Medium	Gibco	A1435101	
Expi293F cells	Gibco	A14527	
Filter tips	USA Scientific	1126-7810	1000 µL
Filter tips	USA Scientific	1120-8810	200 µL
Filter tips	USA Scientific	1120-1810	20 µL
Filter tips	USA Scientific	1121-3810	10 µL
Hypodermic needles	Tyco Healthcare	820112	20 GA x 1-1/2 A
Ice bucket with lid	VWR	10146-184	
JS-5.3 rotor	Beckman Coulter	368690	
Magnesium chloride solution (1 M)	Millipore Sigma	M1028-100ML	
Metal stand and clamp	Fisherbrand	05-769-6Q	

Microcentrifuge tubes	Eppendorf	22600028	1.5 mL
Needle nose pliers			
Optima XE-90 ultracentrifuge	Beckman Coulter	A94471	
Opti-MEM I Reduced-Serum Medium	Gibco	31985062	
OptiPrep density gradient media (iodixanol)	Serumwerk	AXS-1114542	60% iodixanol solution
P1000 Pipet	Gilson	F144059M	
P2 Pipet	Gilson	F144054M	
P20 Pipet	Gilson	F144056M	
P200 Pipet	Gilson	F144058M	
Phenol red solution	Sigma-Aldrich	P0290	
Phosphate buffered saline (PBS)	Sigma-Aldrich	P4474	
Pipet-Aid XP pipette controller	Drummond Scientific	4-000-101	
Plasmid pCapsid	De novo or Addgene, etc.	N/A	We used pACGrh74.
Plasmid pHelper	Addgene	112867	
Plasmid pTransgene	De novo or Addgene, etc.	N/A	We used pdsAAV-GFP.
Pluronic F-68 polyol solution (10%)	Mp Biomedicals	92750049	
Polyethylene glycol 8000	Research Products International	P48080-500.0	
Polyethylenimine HCl Max (PEI-Max)	Polysciences	NC1038561	Dilute in water to 40 μ M
Polypropylene centrifuge tubes, sterile	Corning	431123	500 mL
Polypropylene centrifuge tubes, sterile	Corning	430776	250 mL
Polypropylene Optiseal tubes	Beckman Coulter	361625	
Serological pipettes	Alkali Scientific	SP250-B	50 mL
Serological pipettes	Alkali Scientific	SP225-B	25 mL
Serological pipettes	Alkali Scientific	SP210-B	10 mL
Serological pipettes	Alkali Scientific	SP205-B	5 mL
Shaker flasks	Fisherbrand	PBV1000	1 L
Shaker flasks	Fisherbrand	PBV50-0	500 mL
Shaker flasks	Fisherbrand	PBV250	250 mL
Shaker flasks	Fisherbrand	PBV12-5	125 mL
Sodium chloride solution (5 M)	Fisher Scientific	NC1752640	
Sterile syringes	Fisherbrand	14-955-458	5 mL
Syringe filter	Millipore	SLGV013SL	0.22 micron
Tris-HCl pH 8.5 (1 M)	Kd Medical	RGE3363	
Trypan blue solution	Gibco	15250061	
Tube rack assembly	Beckman Coulter	361646	
Tube spacers (x4)	Beckman Coulter	361669	
Tubing for peristaltic pump	Fisher Scientific	14190516	
Type 70 Ti fixed-angle titanium rotor	Beckman Coulter	337922	
Ultra low temperature freezer			Set at -70 $^{\circ}$ C
Vivaspin 20 centrifugal concentrator	Sartorius	VS2041	
Water bath			Set at 37 $^{\circ}$ C