

Process Development for the Production and Purification of Adeno-Associated Virus (AAV)2 Vector using Baculovirus-Insect Cell Culture System

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Materials

Name	Company	Catalog Number	Comments
1 N Sodium Hydroxide	Sigma-Aldrich	1.09137	For Akta Avant cleaning
2 L flasks	ThermoFisher Scientific	431281	Flask for suspension culture
50 ml Conical tube	ThermoFisher Scientific	14-959-49A	For collection of supernatants
24-well plate	ThermoFisher Scientific	07-200-80	Adherent cell culture plate
250 mL flasks	ThermoFisher Scientific	238071	Flask for suspension culture
Akta Avant 150 with Unicorn Software	Cytiva	28976337	Chromatography system
AVB Sepharose High Performance	Cytiva	28411210	Chromatography medium
Baculovirus-AAV-2 GFP	In-house	non-catalog	AAV transfer vector
Baculovirus-AAV-2 rep-cap	In-house	non-catalog	AAV packaging vector
Nuclease	Sigma-Aldrich	E1014	Enzyme to degrade DNA and RNA
Blocking buffer	Santa Cruz Biotechnologies	516214	Blocking to prevent non-specific antibody binding to cells
Cell lysis buffer	In-house	Non-catalog item	20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5 % Triton X-100
Cellbag, 2 L and 10 L	Cytiva	28937662	Bioreactor bag
Cleaning buffer	In-house	Non-catalog item	100 mM citric acid (pH 2.1)
Cryovial	Thomas Scientific	1222C24	For cryopreservation
DMEM	Sigma-Aldrich	D6429	Growth media for cell lines
Elution buffer	In-house	Non-catalog item	50 mM sodium citrate buffer (pH 3.0)
Ethanol	Sigma-Aldrich	E7073	For disinfection and storage of the chromatography
Filtration unit	Pall Corporation	12941	Membrane filter
HT1080 cell line	ATCC	CCL-121	Fibroblast cell line
HyClone™ SFX-Insect culture media	Cytiva	SH30278.02	Serum-free insect cell growth medium

Peristaltic Pump	Pall Corporation	Non-catalog item	TFF pump
MaxQ 8000 orbital shaker incubator	ThermoFisher Scientific	Non-catalog item	Shaker for suspension culture
Microscope	Nikon	Non-catalog item	Cell monitoring and counting
Mouse anti-baculovirus gp64 PE antibody	Santa Cruz Biotechnologies	65498 PE	Monitoring baculovirus infection in Sf9 cells
Oxygen tank	Praxair	Non-catalog item	40 % Oxygen supply is needed for Sf9 cell growth
PBS	ThermoFisher Scientific	20012027	Wash buffer
Silver Staining kit	ThermoFisher Scientific	LC6100	Staining AAV capsid proteins
Sf9 cells	ThermoFisher Scientific	11496-015	Insect cells
Steile water	In-house	Non-catalog item	For Akta Avant cleaning
Table top centrifuge	ThermoFisher Scientific	75253839/433607	For clarification of Baculovirus supernatant
Tangential Flow Filtration (TFF) cartridge	Pall Corporation	OA100C12	TFF cartridge to concentrate the AAV
Tris-HCl, pH 8.0	ThermoFisher	15568025	Alkaline buffer to neutralize the eluted AAV
WAVE Bioreactor System 20/50	Cytiva	28-9378-00	Bioreactor