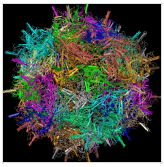

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AAV production for Serotypes with Heparin Binding Capabilities

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Production of Adeno Associated Virus with Heparin Binding Motifs. Adapted from McClure et. al.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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MATERIALS

NAME	CATALOG #	VENDOR
Tris		
Sodium Chloride	PubChem CID: 5234	
Sodium Deoxycholate	PubChem CID: 23668196	
100 ml Polyethylenimine	orb65580	biorbyt
HiTrap Heparin HP affinity column	17040701	Ge Life Sciences
Benzonase® Nuclease	E1014 SIGMA	Sigma-aldrich
DMEM		Invitrogen - Thermo Fisher
Amicon Ultra Centrifugal Filter (100K)	UFC810096	Emd Millipore
Cell scraper	3011	Corning

MATERIALS TEXT

Plasmids Needed Per Prep:

62.5 ug AAV plasmid

125 ug pHelper

62.5 ug pAAV DJ

SAFETY WARNINGS

AAV is BSL1, but should be handled with caution and kept in a hood when processing.

BEFORE STARTING

Plate HEK293T cells to 70-80% confluency in 5 * 15 cm Nunc tissue culture dishes for one batch of virus (enough for injecting 10-20 animals.)

Transfection

1 Mix DNA in 4.5ml MEM as follow

62.5 ug AAV plasmid

125 ug pHelper

62.5 ug pAAV DJ

Filter combined plasmids using 0.22 um filter

2 In a separate tube mix 350ul PEI in 10.5ml MEM (PEI stock solution is 1mg/ml)

3 Wait 5 min then mix the two solution together

4 Incubate for 15 min at RT.

5 Volumize to 100ml with complete DMEM

6 One by one, remove culture medium from each dishes and gently add the fresh transfection solution (20ml for each dishes).

Lysing of cells and harvesting of AAVs

7 48 to 72 hours after transfection (depending on health of the HEK cells), remove media from cell culture plates and discard. The cells will still exhibit nice monolayer formation - of cells start to peel away from the bottom of the flask it is

too late. Watch for media turning from pink to yellow. If media starts to look yellow, it is time to wash.

8 Wash cells with warm (37C) 1x PBS 10ml.

9 Add 20 mL warm PBS to each plate and gently remove cells with cell scraper.

10 Collect suspension in 50 mL falcon tubes.

11 Pellet cells at 800 x g for 10 min

12 Discard supernatant and resuspend pellet in 150 mM NaCl, 20 mM Tris pH 8.0 using 10 mL per culture plate. Split into two 50 mL tubes.

13 Prepare fresh solution of 10% sodium deoxycholate in ddH2O.
Add 1.25 mL of this to each tube for final concentration of 0.5%.
Add benzonase nuclease for final concentration of 50 units/mL.
Mix thoroughly.

14 Incubate at 37 deg C for 1 hour.

15 Remove cellular debris by centrifuging at 3000 x g for 15 min. Transfer supernatant to fresh 50 mL tube and ensure all cell debris has been removed.

16 Freeze supernatant at -20 deg C for 24 hours. (This is important and should not be skipped - it allows for better isolation of virus)

17 Thaw solution, mix thoroughly and incubate at 37 deg C for 1 hour.

18 Centrifuge at 3000 x g for 15 min. Transfer supernatant to fresh 50 mL tube and ensure all cell debris has been removed to avoid blocking heparin columns.

Heparin Column Purification

19 Setup HiTrap heparin columns with peristaltic pump so solutions flow through at 1 mL/minute. Avoid air bubbles in heparin column (and tubes).

20 Equilibrate column with 10 mL 150 mM NaCl, 20 mM Tris, pH 8.

21 Apply 50 mL virus solution to column and allow to flow through.

22 Wash column with 20 mL 100 mM NaCl, 20 mM Tris, pH 8.

23 Using 5 mL syringe continue to wash column with 2 mL 200 mM NaCl, 20 mM Tris, pH 8. Discard flow through.

24 Elute virus into 15 mL centrifuge tube with following steps (Binding affinity of DJ capsid slightly differ from the one of AAV2, see Thomas F. Lerch et al., Strucure 2012).

25 Using 5 mL syringe and gentle pressure, apply the following in order:
1. 1.5mL 300mM NaCl, 20mMTris, pH 8
2. 3mL 350mM NaCl, 20mM Tris, pH 8 3.
1.5mL 450mM NaCl, 20mM Tris , pH8

Concentration and sterile filtration of rAAVs

26 Concentrate vector using Amicon ultra-4 centrifuge with 100000 molecular weight cutoff.
Load 4 mL of column eluate into concentrator and centrifuge at 2000 x g for 2 min at RT.
Discard flowthrough and reload concentrator with remaining virus solution and repeat centrifugation.

27 Concentrated volume should be 250 uL. If concentrated volume is significantly more than this, discard the flow through and continue to centrifuge in 1 min steps until volume is approximately 250 uL.

28 Add 250 uL of PBS to virus for a final volume of 500 uL and remove from concentrator (this step could be skipped if high titer is required).

29 Filter vector through 13 mm diameter 0.2 um syringe, aliquot and store at -80C.