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AAV Purification Kit

Product Insert

Product # 66100

Recombinant adeno-associated virus (AAV) vectors are highly promising tools for both *in vitro* and *in vivo* gene transfer. Norgen's AAV Purification Kit provides a fast and simple procedure for concentrating and purifying AAV vectors from cell lysate and cell culture media. Purification is based on precipitation onto Norgen Biotek's proprietary resin. Contaminating cellular debris is largely removed from the sample via a centrifugation step, while contaminating DNA and RNA is reduced using enzymatic digestion. AAV vector purified in this manner is highly active for use in *in vitro* and *in vivo* transduction experiments.

Norgen's AAV Purification Kit contains sufficient materials for 15 preparations (33.5 mL per prep of supernatant (SN) or a total of 500 mL of supernatant input). Approximately 1 mL of cell pellet can be purified per prep, up to a maximum of 15 mL of cell pellet in total for the entire kit.

Specifications

Kit Specifications	
Resin binding capacity (total per kit)	At least $5x10^{10}$ AAV particles as determined by qPCR
AAV vector serotype	AAV9 and others
Input type	Cells, media
Input volume (AAV supernatant)	1 - 33.5 mL SN per prep (500 mL SN in total)
Input volume (AAV cell pellet)	1 mL cell pellet per prep (15 mL in total)
Minimum elution volume	1 mL per prep
Time to complete purification	2.5 to 4.5 hours with 1 hour hands on time
In vivo transduction	Yes

Kit Components

Component	Product # 63200 (15 samples)
Lysis Solution S	5.5 mL
HL-SAN Nuclease	102 µL
Binding Buffer A	20 mL
Purification Solution C	60 mL
Purification Solution D	130 mL
Wash Solution C	2 x 130 mL
Slurry E	12.5 mL
Elution Buffer O (cap tightly, store at 4°C)	66 mL
Protein Neutralizer	4 mL
Elution tubes (1.7 mL)	50
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Storage Conditions and Product Stability
HL-SAN Nuclease should be stored at -20°C upon arrival. **Elution Buffer O should be stored tightly capped at 4°C** upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. Once opened, the solutions should be stored at 4ºC. This kit is stable for 2 years after the date of shipment.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

AAV vectors are able to transduce humans and animals. Ensure that all hazards are accounted for when working with gene therapy vectors containing potentially hazardous transgenes. Ensure that all appropriate precautions are taken, including using a suitable biosafety cabinet, wearing eye and face protection, wearing gloves, and wearing a face mask or respirator, if applicable. Consult your institutional biosafety committee and NIH guidelines for guidance.

Norgen Biotek is not responsible for any injury or damage which might be due to the failure of the purchaser or any other individual to use this kit or manual in accordance with the specific conditions outlined herein or in accordance with NIH guidelines for Biosafety Level 2 infectious agents and recombinant DNA material.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemical solutions. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Customer-Supplied Reagents and Equipment
- Biosafety cabinet
- Benchtop swinging bucket centrifuge
- Microcentrifuge
- 37°C incubator
- Micropipettors
- 1.7 mL microcentrifuge tubes
- 15 mL conical centrifuge tubes (able to withstand up to ~3,200 x g)
- 50 mL conical centrifuge tubes (able to withstand up to ~3,200 x g)
- pH paper
- Sterile MEM or DMEM media containing phenol red (no serum)

Procedure
All centrifugation steps are carried out in a swinging bucket centrifuge. Please check your centrifuge specifications to ensure proper speed.

Notes prior to use:
- Transfect HEK 293 cells via PEI, calcium phosphate, or alternate methods**. It is recommended that the media be changed 1 day post transfection and replaced with DMEM + L-glutamine in order to reduce the amount of fetal bovine serum proteins left in the media prior to purification.** AAV vector may be harvested 3 to 7 days post transfection. Allowing for a greater amount of time post transfection prior to harvesting may allow for greater accumulation of AAV vector within the cell media. Harvesting only the cell media fraction at this time will allow for easy harvesting of the AAV vector with less contaminating cellular proteins. However, AAV vector may also be purified from the cell pellet fraction alone.
- A swinging bucket centrifuge is highly recommended for maximum kit performance.

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again. HL-SAN Nuclease should be kept on ice during use and should be stored at -20°C.
- **Store Elution Buffer O tightly capped at 4°C with a minimal amount of air left in the bottle.**

1. **For a cell free conditioned media/SN** sample containing AAV (ie, the supernatant fraction), add up to 33.5 mL of sample to a 15 or 50 mL conical centrifuge tube (not provided). Add 1/100 the total volume of sample Lysis Buffer S to the same tube (for example, add 335 µL of Lysis Buffer S to 33.5 mL of sample).

   For a cell pellet, up to 1 mL of cell pellet may be purified per prep by adding 1/50 the pellet volume of Lysis Buffer S and performing freeze-thaw 3 times, alternating between room temperature and a -80°C freezer or liquid nitrogen each time. Next, add 8 pellet volumes of DMEM/MEM media (without FBS or any other additives). Resuspend pellet by pipetting up and down or by vortexing.

2. Add 1/12 the total volume of Purification Solution C to the tube (for example, add 2.8 mL of Purification Solution C to 33.5 mL of supernatant or for a 1 mL cell pellet, add 750 µL of Purification Solution C to the cell pellet/DMEM mixture that is now 9 mL). Mix well by vortexing.

3. Add HL-SAN Nuclease to the AAV sample (2 µL per 10 mL of supernatant, or for the cell pellet mixture from the previous step, 6 µL per 1 mL of starting cell pellet). For example, add 6.7 µL of HL-SAN Nuclease to 33.5 mL of SN or for a 1 mL cell pellet, add 6 µL of HL-SAN nuclease to the mixture from the previous step that is now 9.75 mL. Mix well by inverting tube repeatedly.

4. Digest for 30 minutes to one hour at 37°C or overnight at 4°C.

5. Spin down sample at 3,500 rpm (~2,500 x g) for 10 minutes in order to pellet cell debris. Harvest supernatant. If no visible pellet is observed (for example with a pure supernatant sample) move onto the next step.

6. **(Optional)** Filtration of the sample with a 0.22 µm or 0.45 µm vacuum or syringe driven filter (not provided) may be performed in order to further clarify the sample. A low protein binding filter is recommended.

7. Acidify to pH 3.8 - 4.0 by adding 1.4% Binding Buffer A of the sample volume until a bright yellow color is reached by the DMEM media (containing phenol red pH indicator). pH paper should be used to confirm that the appropriate pH has been reached. Adding Binding Buffer A to a final concentration of approximately 1.4% of total sample volume is generally sufficient to acidify the sample (eg. 469 µL of Binding Buffer A added to a 33.5 mL sample). However, optimization may be required on a case by case basis.

8. Spin down at 3,500 rpm (~2,500 x g) for 8 minutes. Discard pellet and transfer supernatant to a new 15 or 50 mL tube.

9. Add 1/3 the sample volume of Purification Solution D to the tube from the previous step (eg. Add 12.3 mL to a sample that is now ~36.8 mL). Resuspend Slurry E by vortexing or by pipetting up and down until fully resuspended. Add resuspended Slurry E (200 µL Slurry E per 10 mL of total sample volume). Incubate for 20 minutes at 4°C or on ice.

10. Spin down at 4,000 rpm (~3,200 x g) for 1 - 2 hours at 4°C in a spinning bucket centrifuge. For larger volumes (15 - 33.5 mL of input for example), spinning for a longer time may help to recover additional AAV vector. Discard supernatant, being careful not to discard any resin.

11. Wash resin by adding 5 mL of Wash Solution C, vortex for 10 seconds or until resin is fully resuspended, then spin down at 800 rpm (~130 x g) for 2 minutes to pellet resin. Discard supernatant, being careful to leave the resin in the tube.

12. Wash resin a second time by adding 5 mL of Wash Solution C, vortex for 10 seconds or until resin is fully resuspended, then spin down again at 800 rpm (~130 x g) for 2 minutes to pellet resin. Discard supernatant, being careful to leave the resin in the tube.

13. **For a cell pellet** sample, wash resin a third time by adding 5 mL of Wash Solution C, vortex for 10 seconds or until resin is fully resuspended, then spin down again at 800 rpm (~130 x g) for 2 minutes to pellet resin. Discard supernatant, being careful to leave the resin in the tube.

14. For samples that consisted of less than 10 mL of supernatant input, add 250 µL of Elution Buffer O. For other sample types (cell pellet or supernatant input > 10 mL), add 500 µL of Elution Buffer O and vortex for 30 seconds to 1 minute. Ensure that resin is fully resuspended. Spin down at 800 rpm (~130 x g) for 3 minutes. Pipette supernatant into a microcentrifuge tube, being careful to avoid pipetting up excess resin.

15. For samples that consisted of less than 10 mL of supernatant input, add another 250 µL of Elution Buffer O. For other sample types (cell pellet or supernatant input > 10 mL), add another 500 µL of Elution Buffer O and vortex for 30 seconds to 1 minute. Ensure that the resin is fully resuspended. Spin down at 800 rpm (~130 x g) for 3 minutes. Pipette supernatant into the same tube as above, being careful to avoid pipetting up excess resin.

    (Optional) An additional elution can be performed into a separate tube, using 250 µL of Elution Buffer O for samples that consisted of less than 10 mL of supernatant input, or 500 µL of Elution Buffer O for all other sample types. The additional elution step can help recover up to 10-20% of additional AAV.

16. If any resin is remaining in the eluted samples, spin down the sample in a swinging bucket centrifuge at 800 rpm (~130 x g) for 3 minutes or a microcentrifuge at 2,000 rpm (~425 x g) for 1 minute and pipette supernatant into a new tube, being careful not to aspirate resin. Discard tube containing the remaining resin pellet. If necessary, spin down again to collect excess resin and pipette supernatant into a new tube.

17. If desired, the eluate may be pooled into a single tube (if purifying the same vector, for example).

18. Pipette a small amount (<10 µL) of the eluted samples down the length of pH paper. If pH is above 8.5, add a small amount of Protein Neutralizer (10 - 20 µL at a time) until pH is within an acceptable range for storage (pH < 8.5).

19. Filter sterilization through a 0.22 µM or 0.45 µM filter (not provided) is highly recommended prior to use. For a cell pellet sample, filtration can also remove leftover particulates. Optional concentration and buffer exchange with a centrifugal filtration device with a 100K MWCO (such as a Millipore Amicon® Ultra-4 or Ultra-15, 100 KDa MWCO, not included) may be performed after filter sterilization.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor AAV recovery	Incorrect pH adjustment of AAV sample for binding	A pH level of 3.8-4.0 works best for binding AAV to the resin. Ensure that the sample is adjusted to approximately this value with Binding Buffer A prior to sample loading. In phenol red containing media, this will turn the media a bright yellow color.
	Initial titer of sample applied to column was too low	A sufficient amount of AAV particles are required in the starting sample to ensure success in downstream applications. It may be required to increase the volume of starting sample or increase the AAV vector titer of the starting sample by optimizing transfection conditions and/or vector constructs.
	Elution Buffer O stored improperly	Ensure that the elution buffer is tightly capped with a minimal amount of air left in the bottle.

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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