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AAV Purification Midi Kit

Product Insert

Product # 63300

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 spin column chromatography using Norgen's proprietary resin as an ion exchanger. Contaminating cellular debris is largely removed from the sample via a precipitation and 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* transduction experiments, and is eluted into a small volume (1 mL).

Norgen's AAV Purification Midi Kit contains sufficient materials for 4-8 preparations. Each spin column is able to concentrate and purify AAV from 8 mL up to 45 mL of input consisting of cell pellet, cell culture media, or cells and culture media mixed together. The kit may be used to purify up to 8 x 25 mL or 4 x 45 mL of samples using the included columns. Preparation time for 4 samples is approximately 2 to 2.5 hours, with 1.5 hours of hands on time. The kit has a shelf life of 1 year when stored as suggested.

Kit Components

Component	Product # 63300 (4-8 samples)
Lysis Solution S	5.5 mL
DNAse I (store at -20°C)	2 x 25 µL
RNAse A (store at -20°C)	60 µL
Wash Solution C	60 mL
Binding Buffer A	4 mL
Elution Buffer O (store at 4°C)	8.5 mL
Protein Neutralizer	4 mL
Midi Spin Columns (**grey contents**) with Collection Tubes	8
Midi Spin Columns (**white contents**) with Collection Tubes	8
Elution tubes (15 mL)	8
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Storage Conditions and Product Stability

DNAse I and RNAse A 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 4°C. All the reagents should remain stable for at least 1 year in their unopened containers.

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
- 37°C incubator
- Micropipettors
- 1.5 mL microcentrifuge tubes
- 15 or 50 mL conical tubes
- pH paper

Procedure

All centrifugation steps are carried out in a swinging bucket centrifuge. Please check your centrifuge specifications to ensure proper speed. Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4°C, or on ice.

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 may allow for greater accumulation of AAV vector within the cell media. **It is recommended that AAV vector be allowed to accumulate in the cell media until 7 days post transfection, after which point the majority of vector will no longer be cell associated.** Harvesting only the cell media fraction at this time will allow for easy harvesting of the AAV vector, free of contaminating cellular proteins. However**,** if desired, AAV vector may be purified from a mixed cell/cell media sample, the cell media fraction alone, or the cell pellet fraction alone.
- A swinging bucket centrifuge is highly recommended for maximum kit performance.
- Viscous samples or higher input volumes may require additional centrifugation time and higher speeds to fully pass through the column. Filtration of the sample prior to loading onto the column with a 0.2 μm or 0.45 μm filter may improve sample flow through the column during centrifugation.
- 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. The DNAse/RNAse enzymes should be kept on ice during use and should be stored at -20°C.
- **Immediately upon opening, aliquot the Elution Buffer into sterile 1.5 mL microfuge tubes. Store in 1 mL to 1.5 mL aliquots, tightly capped, at 4°C.**

1. Harvesting of Cells and Media containing AAV

a. To purify from a mixed sample containing both cells and cell culture media, harvest the cells directly into the media by pipetting up and down to dislodge the cells. Alternately, a cell scraper can be used to dislodge cells**.**

For a cell culture media only fraction containing no cells, proceed directly to step 1b.

For a sample containing pelleted cells alone, add 16 pellet volumes of DMEM media (no FBS) to cell pellet. The volume of the cell pellet may be estimated using a microcentrifuge tube or micropipettor. Vortex to mix.

b. Add a volume of **Lysis Buffer S** to the sample that is 1/50 of the total sample volume. (eg: add 500 µL of **Lysis Buffer S** to 25 mL of sample). Ensure that all **Lysis Buffer S** is added to the sample as some may remain in the pipette.

c. Add 2 µL **DNAse I** per 10 mL total sample volume (5 µL of **DNAse I** per 25 mL).

d. Add 2 µL **RNAse A** per 10 mL total sample volume (5 µL of **RNAse A** per 25 mL).

e. Mix well by pipetting up and down and by vortexing. Ensure that any cell clumps present are broken up via mixing or pipetting so that efficient lysis can occur.

f. Incubate for 20-40 minutes at 37°C. Mix periodically to ensure even heating.

g. Spin down samples at 3,500 rpm (1,500 x g) for 5 minutes to pellet debris in a swinging bucket centrifuge. If necessary, spin for an additional 5 minutes to pellet debris.

2. DNA Depletion

a. Load supernatant onto **Midi Spin Columns (white contents),** being careful not to disturb the pellet**. DO NOT use Midi Spin Columns (grey contents) as these will be used to bind AAV.** 1-4 mL of supernatant may be loaded at a time onto the **Midi Spin Columns (white contents)**. Spin at 2,000 rpm (500 x g) for 3-5 minutes in a swinging bucket centrifuge. If liquid is remaining in column, repeat for an additional 3-5 minutes. **Collect flowthrough and store** in sterile 15 mL or 50 mL centrifuge tubes. Repeat loading as needed until all samples pass through the columns. 4 to 45 mL may be processed per column. Discard **Midi Spin Columns (white contents)** after use.

3. Acidification of Sample

a. Optimal binding of AAV to the column occurs at pH 3.8. Gradually acidify the sample using **Binding Buffer A** 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 1.3% of total sample volume is generally sufficient to acidify the sample (eg. 325 µL of **Binding Buffer A** added to a 25 mL sample). However, optimization may be required on a case by case basis. It is recommended that **Binding Buffer A** be added slowly and with gentle mixing so as to avoid localized regions of low pH.

b. Spin down the sample at 3,500 rpm (1,500 x g) for 8-10 minutes. A swinging bucket centrifuge is highly recommended. Harvest the supernatant, being careful not to aspirate the pelleted cell debris. If necessary, spin for an additional 10 minutes to pellet debris.

4. Midi Spin Column (grey) Activation

a. Place provided **Midi Spin Column (grey contents)** into collection tube.

b. Activate column by applying 0.5 mL of **Wash Solution C** to column, then spin at 2,000 rpm (500 x g) for 1 minute in a swinging bucket centrifuge. Discard flowthrough.

5. AAV Vector Binding

a. Begin purification by loading 1-5 mL of pH adjusted sample into **Midi Spin Column (grey contents)** and spinning at 2,000 rpm (500 x g) for 2 minutes in a swinging bucket centrifuge. Spin duration can be increased to 3-5 minutes if liquid is still remaining in the spin column. Discard flowthrough.

b. Repeat loading of 1-5 mL of pH adjusted sample to **Midi Spin Column (grey contents)** as needed until all liquid passes through the column via centrifugation at 2,000 rpm (500 x g) for 2 minutes. Up to 45 mL may be processed in this way.

6. Column Wash

a. Once the entire sample has passed through the column, wash **Midi Spin Column (grey contents)** by applying 2 mL of **Wash Solution C** and spinning at 2,000 rpm (500 x g) for 3 minutes. Discard flowthrough.

b. Repeat wash step by applying 2 mL of **Wash Solution C** to column and spinning at 2,000 rpm (500 x g) for 3 minutes. Discard flowthrough.

c. Conduct a dry spin to eliminate remaining liquid by spinning at 3,500 rpm (1,500 x g) for 2 minutes.

d. Perform a pre-elution step by adding 30 µL of **Elution Buffer O** to column. Spin at 3,500 rpm (1,500 x g) for 2 minutes. Discard flowthrough.

6. AAV Vector Elution

a. Transfer **Midi Spin Column (grey contents)** to an elution tube.

b. Elute sample by applying 1 mL of **Elution Buffer O** to column. Spin at 800 rpm (80 x g) for 4 minutes followed by 3,500 rpm (1,500 x g) for 2 minutes.

c. Add 40 µL of Protein Neutralizer to the 1 mL of eluted virus. Add gradually and with mixing to avoid localized regions of low pH.

d. A small amount of resin may have become dislodged during the elution process. If this occurs, the elution can be spun down briefly at 2,000 rpm (500 x g) for 3 minutes in a swinging bucket rotor to pellet the resin and the supernatant can be transferred to a new tube. Eluted vector may be stored at 4°C or -20°C for short term storage. -80°C is highly recommended for long term storage. Avoid repeated freeze thaw cycles as vector aggregation may occur. Store in single use aliquots if possible.

e. Store unused elution buffer as described in the introduction, minimizing air exposure by aliquoting elution buffer into microfuge tubes (1 mL to 1.5 mL aliquots) and capping tightly. Store at 4°C.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
AAV sample does not flow through the column	Centrifugation speed was too low	Check the centrifuge to ensure that it is capable of generating 1,500 x g. Sufficient centrifugal force is required to move the liquid phase through the resin.
	Inadequate spin time	Spin an additional two minutes to ensure that the liquid is able to flow completely through the column.
	Cell debris obstructing column	Ensure that no cell debris is inadvertently applied to column following lysate centrifugation. Care should be taken to ensure that only the supernatant is applied to the column. Filtration of supernatant through a 0.2 µm or 0.45 µm filter prior to loading may decrease clogging of the column.
Poor AAV recovery	Incorrect pH adjustment of AAV sample	A pH level of 3.8 works best for binding AAV to the column. 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 stored improperly	Ensure that the elution buffer is aliquoted into 1.5 mL microfuge tubes after opening, taking care to minimize the amount of air left in the tubes (1 mL to 1.5 mL aliquots). Store tightly capped at 4°C.
Pellet observed following elution	Too high input loaded	If a pellet is observed following elution, the input may have been too high for a single column. Spin down elution containing pellet, and harvest supernatant, as AAV vector should mostly be present in the supernatant. The pellet can be re-inputed into a fresh column, and the cell pellet protocol used to harvest remaining AAV present in the pellet.

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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