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AAV Purification Maxi Slurry Kit

Product Insert

Product # 63250

Recombinant adeno-associated virus (AAV) vectors are highly promising tools for both *in vitro* and *in vivo* gene transfer. Norgen's AAV purification kits provide 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 and column purification. AAV vector purified in this manner is highly active for use in *in vitro* transduction experiments, and is eluted into a small volume (1-10 mL) using the optional concentration step.

Norgen's AAV Purification Maxi Slurry Kit contains sufficient materials for 1-10 preparations. Each spin column is able to concentrate and purify AAV from 45 mL to 90 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 1 x 900 mL samples or 10 x 45-90 mL samples using the included columns. Preparation time for 1 x 900 mL sample is approximately 2.5 to 3.5 hours, with an optional concentration step requiring an additional 30 min.

Kit Components

Component	Product # 63250 (1-10 preps)
Lysis Solution S	20 mL
DNAse I (store at -20°C)	210 µL
RNAse A (store at -20°C)	240 µL
Wash Solution C	3 x 60 mL
Binding Buffer A	2 x 8 mL
Slurry E	2 x 14.5 mL
Elution Buffer O (store at 4°C)	66 mL
Protein Neutralizer	4 mL
Maxi Spin Columns **(white contents)** inserted into Collection Tubes	10
Maxi Spin Columns **(grey contents)** inserted into Collection Tubes	10
Midi Spin Columns **(grey contents)** inserted into Collection Tubes	10
Elution tubes (50 mL)	10
Midi Elution tubes (15 mL)	10
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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
- Microcentrifuge
- 37°C incubator
- Micropipettors
- 1.5 mL microcentrifuge tubes
- 15 mL conical centrifuge tubes
- 50 mL conical centrifuge tubes
- 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, relatively free of contaminating cellular proteins. However**,** AAV vector may also be purified from a mixed cell/media sample, the cell media fraction alone, or the cell pellet fraction alone. Harvesting both the cells and media at the same time in a mixed sample allows for maximum yield to be achieved but may release more cellular proteins.
- 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.

- **Store elution buffer O tightly capped at 4°C. Whenever possible, minimize exposure to air by aliquoting into sterile smaller containers with a minimal amount of air left in container.**

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. Divide the sample into 45 mL aliquots in clean, sterile 50 mL conical bottom centrifuge tubes. Add a volume of **Lysis Buffer S** to the sample that is 1/50 of the total sample volume. (eg: add 900 µL of **Lysis Buffer S** to 45 mL of sample). Ensure that all **Lysis Buffer S** is added to the sample as some may remain in the pipette.

c. Add 10 µL **DNAse I** per 45 mL of sample.

d. Add 10 µL **RNAse A** per 45 mL of sample.

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 30 minutes to 1.5 hours at 37°C. Mix periodically to ensure even heating and to break up cell clumps.

g. Spin down the samples at 3,500 rpm (1,500 x g) for 5 minutes. A swinging bucket centrifuge is highly recommended.

2. Plasmid DNA and gDNA Depletion with Maxi Spin Columns (white contents)

a. Load supernatant onto **Maxi Spin Columns (column with white contents), in 50 mL tube,** being careful not to disturb the pellet**. DO NOT use maxi or midi columns with grey contents as these will be used to bind AAV.** 20-22 mL of sample may be loaded at a time onto the **Maxi 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 45 mL aliquots in sterile 50 mL centrifuge tubes. Repeat loading as needed until all samples pass through the columns. Up to 90 mL may be processed per column. Discard **Maxi Spin Columns (with white contents)** after use.

3. Acidification of Sample

a. Optimal binding of AAV to the column occurs at pH 3.8. Gradually acidify the DNA-depleted 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. 585 µL **Binding Buffer A** added to a 45 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 minutes. A swinging bucket centrifuge is highly recommended.

c. Transfer supernatant by pouring or pipetting into new 50 mL tubes, being careful to avoid transfer of any pelleted material.

4. Column Activation for Maxi Spin Columns (grey contents)

- **a.** Obtain a **Maxi Spin Columns (with grey contents)** inserted into a collection tube (50 mL tube).
- **b.** Activate **Maxi Spin Columns (with grey contents)**, **in 50 mL tube** by applying 1 mL of **Wash Solution C** to columns, then spin at 2,000 rpm (500 x g) for 1 minute in a swinging bucket centrifuge. Discard flowthrough.

5. AAV Vector Binding

- **a.** Thoroughly resuspend **Slurry E** by vortex mixing or inverting repeatedly. Add 150 µL of **Slurry E** per 10 mL of supernatant from step 3 (675 µL for 45 mL volume of sample). Place in an orbital shaker at 300 rpm. The temperature may be between room temperature and 37°C, if the shaker is located in an incubator or warm room. Shake for 20-40 minutes.
- **b.** Load the sample containing the **resuspended** slurry onto multiple **Maxi Spin Columns (with grey contents),** if necessary. Users may load up to 20-22 mL of mixed slurry and sample onto each column and then spin at 2,000 rpm (500 x g) for 3-5 minutes in a swinging bucket centrifuge. Spin duration can be increased an additional 5-10 minutes if liquid is still remaining in the spin columns. The resin will come to rest on top of the filter within the column. Leave resin in place and discard flowthrough. 90 mL of sample may be loaded per maxi column.

  Note: The flowthrough can be saved for later analysis or purification if initial loading was unsuccessful.

- **c.** Repeat loading of 20-22 mL of sample to **Maxi Spin Columns (grey contents)** as needed until all liquid passes through the column(s) via centrifugation at 2,000 rpm (500 x g) for 2 minutes. If the resin has settled at the bottom of the sample tube, briefly **resuspend** by pipetting up and down or vortexing. Up to 90 mL may be processed in this way, per column. Ten columns are provided, allowing the user to process a 900 mL combined input per kit.

6. Column Wash

- **a.** Once the entire sample has passed through the column, wash each **Maxi Spin Column (grey contents)** by applying 5 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 5 mL of **Wash Solution C** to each column and spin at 2,000 rpm (500 x g) for 3 minutes. Discard flowthrough.
- **c.** Conduct a dry spin to eliminate remaining liquid by spinning at 2,000 rpm (500 x g) for 3 minutes. Discard flowthrough.
- **d.** Perform AAV vector pre-elution by applying 200 µL of **Elution Buffer O** to each column. In order to ensure that **Elution Buffer O** reaches all of the surfaces of the resin, briefly vortex the column in its tube for 5-10 seconds per tube after adding **Elution Buffer O,** until the resin is resuspended. Spin at 2,000 rpm (500 x g) for 3 minutes.

  Note: Store **Elution Buffer O** as described in introduction, taking care to cap tightly and minimizing exposure to air.

7. AAV Vector Elution

- **a.** Transfer **Maxi Spin Columns (grey contents)** to elution tubes (50 mL centrifuge tube).
- **b.** Elute sample by applying 4 mL of **Elution Buffer O** to each column. In order to ensure that **Elution Buffer O** reaches all of the surfaces of the resin, briefly vortex the columns in their tubes for 5-25 seconds per tube after adding **Elution Buffer O,** until the resin is resuspended. Spin at 2,000 rpm (500 x g) for 5 minutes to elute the AAV vector.
- **c.** Add 50 µL of **Protein Neutralizer** to each 4 mL of eluted vector. Add gradually and with mixing to avoid localized regions of low pH.
- **d.** AAV vector may be pooled and used as is for downstream applications. 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. If not utilizing a further concentration step, 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 freeze thaw cycles as vector aggregation may occur. Store in single use aliquots whenever possible. If additional concentration is desired, proceed to the optional concentration protocol described below.

Appendix A: Optional Concentration of Eluted AAV

An optional protocol for further concentration of the eluted sample is described below. A centrifugal filtration device with 100K MWCO (such as a Millipore Amicon® Ultra-4 or Ultra-15, not included) may also be used instead of, or in addition to, the included concentration protocol to provide additional selectivity based on the molecular weight cutoff of the filtration device, or to concentrate the eluted vector. Other alternatives such as tangential flow filtration or dialysis may also be used to concentrate the AAV vector or to perform additional purification.

Concentration with Midi Spin Columns (optional)

5-10 mL of eluted vector from the previous step may be loaded onto a **Midi Spin Columns (grey contents)** to concentrate a further 3-10 fold. As the final elution volume for the Midi Spin Column is 1 mL, using 4 midi columns to concentrate would result in a final elution volume of 4 mL. It may be preferable to use the full amount of provided midi columns in order to avoid overloading of the resin for high input samples, but fewer columns may be used if a lower final volume is desired.

1. **Addition of DMEM and Lysis Buffer S**
   - **a.** To each elution from the previous slurry/Maxi column purification, add 0.25X of the eluted sample volume of **MEM or DMEM** media containing phenol red indicator (no FBS) to the samples (eg. Add 1 mL of **MEM/DMEM** media to every 4 mL of the elution from the previous step).
   - **b.** To each elution from the previous slurry/Maxi column purification, add 1/50 of the eluted sample volume of **Lysis Buffer S** to the samples (eg. Add 0.08 mL of **Lysis Buffer S** to every 4 mL of samples now also containing MEM/DMEM).

2. **Acidification of Samples**
   - **a.** Adjust the pH of each sample to ~3.8. Gradually acidify the sample using **Binding Buffer A** until a light yellow color is reached by the MEM/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 volume of approximately 1.8% of the total sample volume is generally sufficient to acidify the sample (eg. 72 µL **Binding Buffer A** added to every 4 mL of eluted 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.

3. **Midi Spin Column (grey contents) Activation**
   - **a.** Place provided **Midi Spin Columns (grey contents)** into 15 mL collection tubes.
   - **b.** Activate **Midi Spin Columns (grey contents)** by applying 500 µL of **Wash Solution C** to columns, and then spin at 2,000 rpm (500 x g) for 1 minute in a swinging bucket centrifuge. Discard flowthrough.

4. **Apply the Acidified Sample to Midi Spin Columns (grey contents)**
   - **a.** Between 4 and 10 mL of sample from the previous steps may be loaded onto the **Midi Spin Columns (grey contents)** for concentration. Up to 4 mL of pH adjusted sample may be loaded per Midi Column per spin. Spin 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 up to 4 mL of pH adjusted sample per column as needed until all liquid passes through the column via centrifugation at 2,000 rpm (500 x g) for 2 minutes.

5. **Column Wash**
   - **a.** Once the entire sample has passed through the column, wash the columns 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 columns 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 each column. Spin at 3,500 rpm (1,500 x g) for 2 minutes.

     Note: Store **Elution Buffer O** 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.

6. **AAV Vector Elution**
   - **a.** Transfer **Midi Spin Columns (grey contents)** to elution tubes (15 mL centrifuge tubes).
   - **b.** Elute samples by applying 1 mL of **Elution Buffer O** to each column. Spin at 800 rpm (80 x g) for 4 minutes followed by 3,500 rpm (1,500 x g) for 1 minute.
   - **c.** Check the pH of a small amount of the eluted virus with pH paper. A small aliquot (10-20 µL) of sample can be pipetted down the length of pH paper to determine pH. If pH is above 8.5, add 40 µL of **Protein Neutralizer** to the 1 mL of elution from each column. Add gradually and with mixing to avoid localized regions of low pH. If below 8.5, proceed to the next steps.
   - **d.** An additional elution (optional) can be carried out by transferring the **Midi Spin Columns (grey contents)** to a new elution tube (not provided) and repeating **Step 6b** which may recover an additional 10-20% of vector in a separate elution. Adjust pH with Protein Neutralizer as described in **Step 6c.**
   - **e.** 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. The eluted vector may be pooled at this point for quantification and storage. It may be advisable to pool the first and second elutions separately so as not to dilute the first elution with the second. 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.

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.

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 may decrease clogging of the column. The sample can also be divided amongst several columns.

Problem	Possible Cause	Solution and Explanation
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.

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