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Adenovirus Purification Kit

Product Insert

Product # 67600

Adenoviral vectors are useful tools for both *in vitro* and *in vivo* gene transfer, and oncolytic viruses based on adenovirus are highly promising for cancer treatment. Norgen's Adenovirus Purification Kit provides a fast and simple procedure for concentrating and purifying adenoviral 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 centrifugation step, while contaminating DNA and RNA is reduced using enzymatic digestion. Adenoviral vectors purified in this manner are highly active for use in transduction experiments.

Norgen's Adenovirus Purification Kit contains sufficient materials for 15 preparations (33.5 mL per prep of supernatant 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 (per prep)	At least $3x10^{11}$ adenovirus particles as determined by qPCR At least $3x10^{8}$ transducing units as determined by transduction assay
Input type	Cells, media
Input volume (Supernatant)	1 - 33.5 mL SN per prep (500 mL SN in total)
Input volume (Cell pellet)	1 mL cell pellet per prep (15 mL in total)
Minimum elution volume	1.3 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 # 67600 (15 preps)
Lysis Buffer 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 P (cap tightly, store at 4°C)	66 mL
Protein Neutralizer	4 mL
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability
HL-SAN Nuclease should be stored at -20°C upon arrival. Elution Buffer P should be stored tightly capped at 4°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature. 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.

Adenoviruses and adenoviral vectors are able to infect humans and animals. Ensure that all hazards are accounted for when working with gene therapy vectors, oncolytic viruses, or potential human or animal pathogens. 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 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 chemicals. 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 benchtop microcentrifuge or swinging bucket centrifuge at specified speeds. Please check your microcentrifuge specifications to ensure proper speed.

Notes prior to use:
- Actively growing HEK293 cells may be infected at approximately 90% confluency with the adenoviral stock or isolate of interest, under typical cell culture conditions. Cells may be grown in DMEM media containing 2-4 mM L-glutamine, 10% fetal bovine serum with appropriate antibiotics. Just prior to infection with the adenovirus stock or isolate, media may be removed and replaced with DMEM plus L-glutamine, without fetal bovine serum in order to reduce the amount of fetal bovine serum proteins in the final elution.
- Infection of 293 cells with adenovirus may need to be optimized by varying the MOI across a range of values in order to maximize final adenovirus yield
- Observe 293 cells daily following adenovirus infection. Cells should be incubated for a minimum of 36 hours prior to harvesting. Once 70% of cells exhibit visible cytopathic effects (CPE), a cell scraper, or sterile rubber policeman can be used to dislodge cells into the media. Alternatively, pipetting up and down with a 5 or 10 mL pipette is often

sufficient to dislodge cells into the medium. Harvest cells and media and store in sterile 50 mL centrifuge tubes at -80°C if not using immediately.

- If adenovirus vectors are being produced via transfection with recombinant plasmids/bacmids encoding adenoviral components, the media can be changed one day post transfection and be replaced with DMEM + L-glutamine in order to remove fetal bovine serum proteins and excess non-internalized plasmids/bacmids.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Elution Buffer P may be used at 4°C or room temperature but should be stored at 4°C. Ensure that all other 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 HL-SAN enzyme should be kept on ice during use and should be stored at -20°C.
- **Store Elution Buffer P tightly capped at 4°C**

1. **For a cell free conditioned media/SN** sample containing adenovirus (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 sample volume of 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** sample 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 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 adenovirus sample (2 µL per 10 mL of **total** supernatant sample volume, 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 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 **total** 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. 508 µL of Binding Buffer A added to a 36.3 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 **total** sample volume of Purification Solution D to the tube from the previous step (eg. Add 12.3 mL to a sample that is now ~37 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 adenovirus vector. Discard supernatant, being careful not to discard any resin.

11. Wash resin by adding 5 mL of Wash Solution C, vortex briefly for 3-5 seconds to resuspend resin, 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 3-5 seconds to resuspend resin, 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 3-5 seconds to resuspend resin, 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. Conduct a pre-elution to prepare the adenoviral vector for elution from the resin by adding 200 µL of Elution Buffer P and vortexing for 10 to 30 seconds. Ensure that resin is fully resuspended. Spin down at 800 rpm (~130 x g) for 3 minutes. Discard supernatant, being careful to leave the resin in the tube.

15. For samples that consisted of less than 10 mL of supernatant input, add 300 µL of Elution Buffer P. For other sample types (cell pellet or supernatant input > 10 mL), add 650 µL of Elution Buffer P and vortex for 10 to 30 seconds. 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.

16. For samples that consisted of less than 10 mL of supernatant input, add another 300 µL of Elution Buffer P. For other sample types (cell pellet or supernatant input > 10 mL), add another 650 µL of Elution Buffer P and vortex 10 to 30 seconds. 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 300 µL of Elution Buffer P for samples that consisted of less than 10 mL of supernatant input, or 650 µL of Elution Buffer P for all other sample types. The additional elution step can help recover additional adenovirus.

17. 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.

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

19. 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).

20. 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 adenovirus recovery	Incorrect pH adjustment of adenovirus sample	A pH level of 3.8-4.0 works best for binding adenovirus 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 adenovirus 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 adenovirus vector titer of the starting sample by optimizing transfection conditions and/or vector constructs.
	Elution Buffer P stored improperly	Ensure that the elution buffer is tightly capped with a minimal amount of air left in the bottle. Store at 4°C.

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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