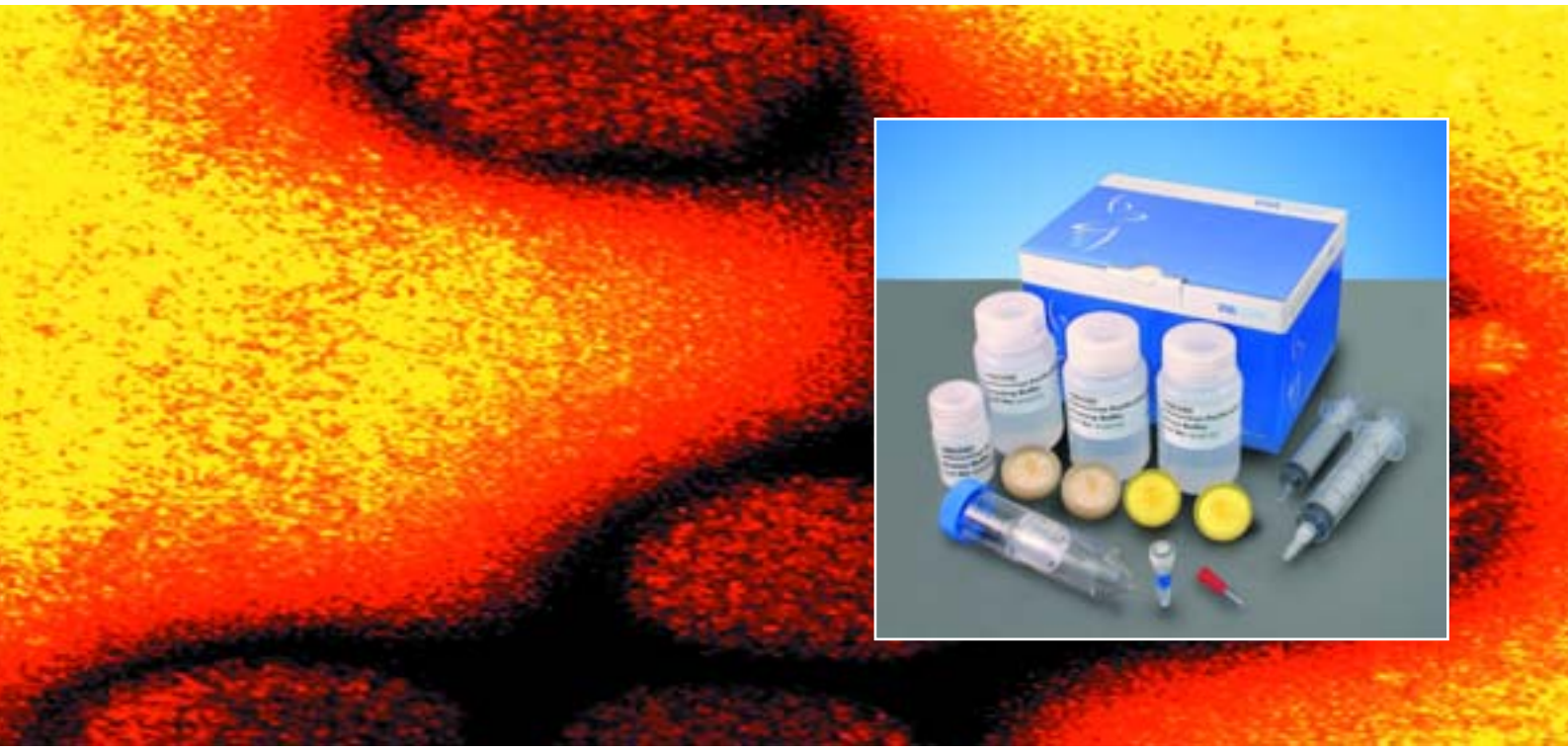
VIVASCIENCE
sartorius group

Vivapure® AdenoPACK™ 100

A kit for Adenovirus purification and concentration



Introduction

The AdenoPACK 100 adenovirus purification and concentration kit is intended for researchers who need to recover a range of 1 - 5 x 10^{12} purified virus particles in a milliliter for in-vitro transfections. The kit includes all reagents and devices necessary for clarification, purification and concentration of Adenovirus Typ 5 from HEK293 cell cultures.

The rapid two-hour purification is made possible by use of Vivascience's membrane adsorber technology, unique in its capability to efficiently capture and recover large virus particles. When compared to chromatography media, membrane adsorbers provide large 3000 nm pores allowing unrestricted access and recovery of Adenovirus from the charged adsorber surface.

Convective flow through the AdenoPACK syringe filter devices provides high-speed separations not possible with chromatography and cesium chloride density gradients. Moreover, Adenovirus is recovered in non-cytotoxic buffers.

AdenoPACK 100 Adenovirus purification and concentration kit features

Feature		Benefit
Virus purification based on membrane adsorber technology	→	High-speed process completed in about 2 hours Excellent viral access provides quantitative recoveries
Kit contents include Minisart, AdenoPACK syringe filters, Vivaspin and all buffers	→	Convenient, easy to use alternative
Able to process 1 to 5 plates (20 to 100 ml)	→	Flexible

Vivapure AdenoPACK™ 100 for Adenovirus purification and concentration

Protocol for Virus purification

The Vivapure AdenoPACK 100 protocol is simple and fast to perform and uses the following steps to concentrate and purify adenovirus type 5 strains.

A. Sample Preparation

1. Infect HEK 293 cells with adenovirus stock until most show cytopathic effects. Harvest cells and lyse by freeze / thaw cycles. Remove cell debris by centrifugation.
2. Digest nucleic acids with Benzonase®* and filter the Benzonase® treated supernatant and dilute with loading buffer.

B. Sample Loading

Pass the prepared supernatant slowly through the AdenoPACK units. Use a single unit for up to 60 ml virus culture or use both units in tandem for up to 100 ml virus culture.

C. Washing

Wash away residual medium, contaminating proteins and nucleic acids with washing buffer.

D. Elution

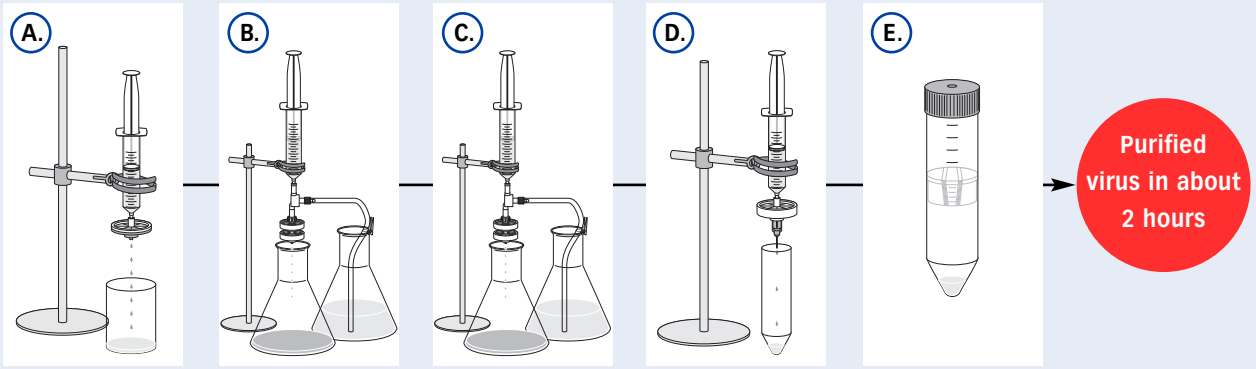
Elute purified viral particles with elution buffer using a special elution tip to aid in judging the correct flow rate.

E. Final concentration / buffer exchange

Virus concentration may be increased using Vivaspin 15R centrifugal concentrators. If desired, Vivaspin 15R may also be used to exchange elution buffer for appropriate physiological or storage buffer.

Handling Overview - Purified virus in about 2 hours

The AdenoPACK Adenovirus purification and concentration kit contains all materials necessary to obtain a range of 1 - 5 x 10^{12} virus particles in a milliliter.



Add Benzonase® and clarify viral supernatant | Load cleared viral supernatant | Wash | Elute | Concentrate

Benzonase® is a trademark of Merck KGaA, Darmstadt, Germany.

Vivapure AdenoPACK™ 100 for Adenovirus purification and concentration

AdenoPACK Membrane Adsorbers

The Sartorius AG / Vivascience AG ion exchange membrane adsorber technology used in AdenoPACK encompasses the advantages of open structures of membranes with the benefits of classical chromatography. Our membrane adsorbers with porous matrices, high capacities, low differential pressures, high flow rates and low unspecific adsorption show an excellent performance in small scale virus purification. However, they are also scalable and confirm to cGMP facilities to large volume, high performance separation, reducing the processing time by a factor of 10 in the final process.

Typical performance

For a normal yielding vector, 5 x 15 cm culture plates purified using this method yield a range of 1 - 5 x 10^{12} viral particles.

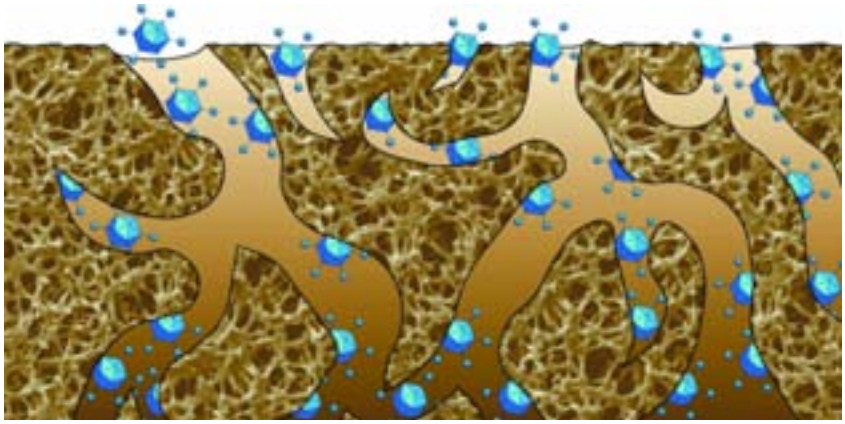
Fluorescent cell assay

Recombinant Adenovirus-GFP eluate, purified with AdenoPACK 100 membrane adsorber technology, was used to infect HeLa cells. As the construct contains a GFP gene, successfully transfected cells can be easily identified by fluorescence microscopy. Cell viability and infection rate, determined by microscopy indicate the high purity of the virus concentrates and the levels of residual endotoxins allow direct use in subsequent in vitro applications.

Virus purification tests conducted in cooperation with Progen Biotechnik GmbH, Heidelberg.

PROGEN

Providing custom made vector development and production service for Adenovirus and Adeno-associated Virus vector systems.

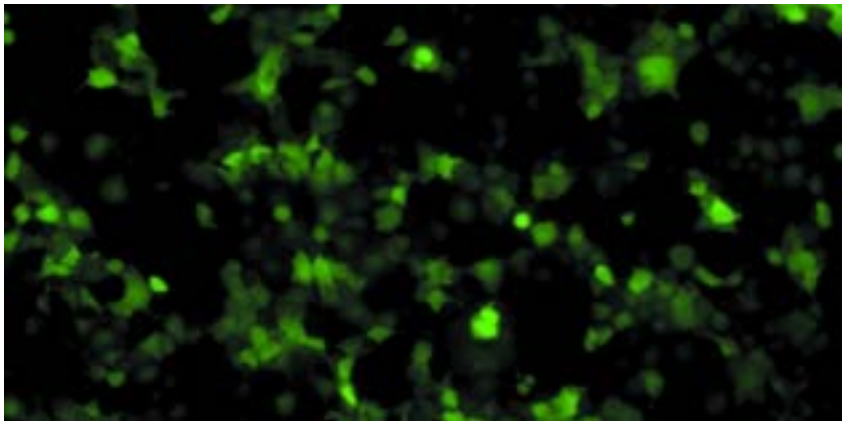


Membrane adsorbers are ideal for virus purification. Large flow through pores allow unrestricted adenovirus access and convective transport speeds purification.

Purification results from preparations with Ad5 GFP-constructs

Purification method	Process time	Eluate	Recovery**	Viral Particles
60 ml culture	1 - 2 hours	1 ml	65%	1 - 2 x 10^{12}
100 ml culture	1 - 2 hours	1 ml	85%	2 - 5 x 10^{12}
CsCl	24 - 48 hours	1 - 2 ml*	60 - 70%	$10^{11\text{-}12}$

**before dialysis* ***before buffer exchange*



Photography: kindly provided by Dr. Lux, University Hospital and University of Applied Sciences, Mannheim. COS-1 cells infected with Ad5 GFP-constructs after purification and concentration with Vivapure AdenoPACK 100.

Kit Specifications	
Sample Size	20 to 100 ml of Adenovirus supernatants
Virus particles (VP) per ml	Typically 1 - 5 x 10^{12}
VP/IU	20 - 50
Processing time	Typically 2 hours

Ordering information

Materials of construction	
AdenoPACK syringe filter housing:	Polysulfon
AdenoPACK syringe filter membrane	Stabilized regenerated cellulose
Minisart housing	Cryolite
Minisart Plus membrane	Cellulose acetate with glass fiber prefilter
Vivaspin housing	Polycarbonate
Vivaspin membrane	Hydrosart stabilized regenerated cellulose
Buffer containers	LDPE

Kit Contents and ordering information	
AdenoPACK 100	VS-AVPQ101
AdenoPACK Membrane Adsorber	2
Benzonase®* 12.5 U/µl	120 µl
Minisart Plus	4
20 ml Syringe	4
Tubing set and one way vale	1
10 ml syringe (elution)	2
Elution Tip (Red)	2
Loading Buffer	1 x 120 ml
Washing Buffer	1 x 120 ml
Elution Buffer	1 x 20 ml
Vivaspin 15R concentrator	4
Instructions	1 each for Kit and Vivaspin
*Benzonase® must be stored at -20°C upon receipt	

Warning: The virus purified using this kit is capable of infecting human or animal cells and could, depending on the gene insert, expose the user to potentially hazardous biological material. Adenoviruses have been designated as Level 2 biological agents. All protocols detailed in these operating instructions must be performed under at least Biosafety Level 2 working condition this kit is NOT intended for human or animal diagnostic or therapeutic applications.

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