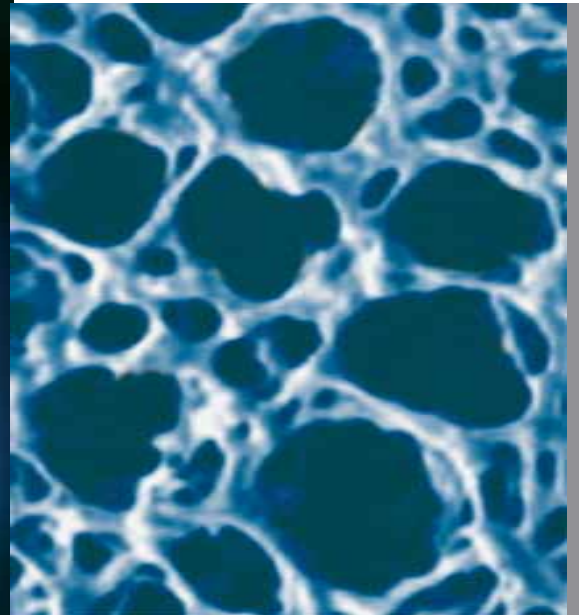




**VIVASCIENCETECHNOLOGY**



**Vivapure<sup>®</sup>**  
**AdenoPACK<sup>™</sup>**  
**20 | 100 | 500**  
for Adenovirus  
Purification and  
Concentration

# AdenoPACK Advantages

## Fast and easy virus purification

- Purification completed in 2 hours
- Convenient, over 10 × faster alternative to CsCl density gradient

## Quantitative yields

- In contrast to CsCl density gradient, the complete cell culture is used for virus purification and not only the viral pellet

## Flexible product range

- Applicable from initial construct screening to large scale virus production

## Complete Kit

- Including filtration devices, AdenoPACK Units for virus purification, Vivaspin and all buffers

## Low endotoxin levels

- High cell viability and infection rates due to endotoxin levels of < 0.025 EU/ml



### Fast and easy to use kits for adenovirus purification

Recombinant adenovirus vectors are the preferred method for a wide range of gene delivery applications. The AdenoPACK adenovirus purification and concentration kits offer researchers who need to recover up to  $3 \times 10^{13}$  purified recombinant adenovirus particles for in-vitro transfection a fast, safe and easy to use solution. The kits include all reagents and devices necessary for clarification, purification and concentration of adenovirus type 5 from HEK293 cell cultures in only two hours. These straight forward kits replace time-consuming and labor-intensive 48 hour CsCl density gradients.

### Rapid virus purification by Membrane Chromatography

The Sartorius AG ion exchange membrane adsorber technology used in AdenoPACK is unique in its capability to efficiently and rapidly 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 traditional chromatography and cesium chloride density gradients.

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.

### Three different kit sizes to meet every need

AdenoPACK kits are offered as AdenoPACK 20, AdenoPACK 100 and AdenoPACK 500, for the purification and concentration of adenovirus type 5 from 20 ml to 500 ml cell culture, leading to  $1 \times 10^{11}$  -  $3 \times 10^{13}$  purified viral particles. For each sample volume, the most convenient handling method is offered for ultimate convenience. To this end, preparations using AdenoPACK 20 are pursued in spin column format in a centrifuge, AdenoPACK 100 is a manually operated kit in syringe filter format\*, and AdenoPACK 500 is a pump driven kit.

\* Vivapure AdenoPACK 100 can optionally be operated with a laboratory pump and an infusion pump, for which protocols are provided on our web page [www.sartorius.com](http://www.sartorius.com). Additionally, the tubes and adaptors needed for these operation modes can be ordered.

### Purification results from preparations with Ad5 GFP-constructs

Purification method	Process time	Eluate	Recovery***	Viral Particles
AdenoPACK 20 / 20 ml culture	1 hour	1 ml	65-70 %	$1 \times 10^{11-12}$
AdenoPACK 100 / 60 ml culture	1 - 2 hours	1 ml	65%	$1 - 3 \times 10^{12}$
AdenoPACK 100 / 200 ml culture	2 hours	1 ml	80%	$1 \times 10^{13}$
AdenoPACK 500 / 500 ml culture	2 hours	1 ml	80%	$1 - 3 \times 10^{13}$
500 ml CsCl density gradient	24 - 48 hours	1 - 2 ml**	60 -70%	$1 \times 10^{11-12}$

\*\*after dialysis

\*\*\*before buffer exchange



Sample Preparation 45 min.



Sample Loading 30 min.

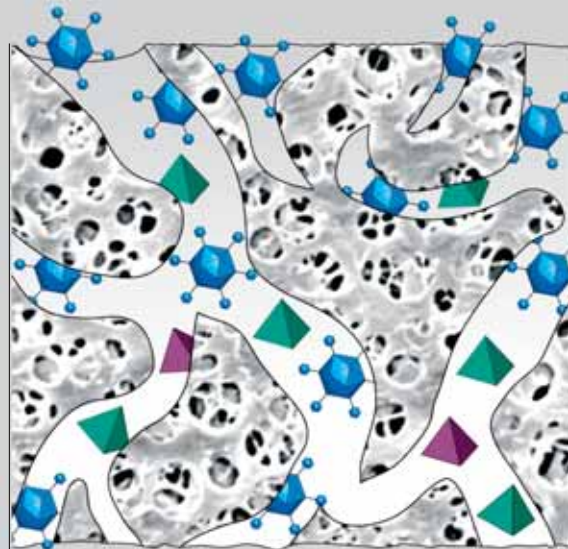


Washing 10 min.

**Easy to follow and fast protocol for virus purification.** The Vivapure AdenoPACK protocols are simple and fast to perform and use the following steps to complete the process.

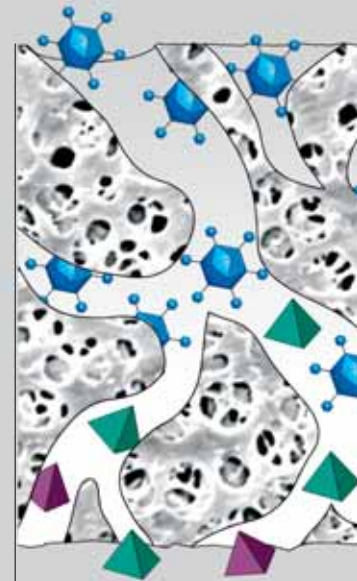
#### A. Sample Preparation

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®, filter the Benzonase treated supernatant and dilute with 10x loading buffer.



#### B. Sample Loading

Pass|centrifuge the prepared supernatant through the Adeno-PACK units. When using Adeno-PACK 100, use a single unit for up to 60 ml virus culture or use both units in tandem for up to 200 ml virus culture.



#### C. Washing

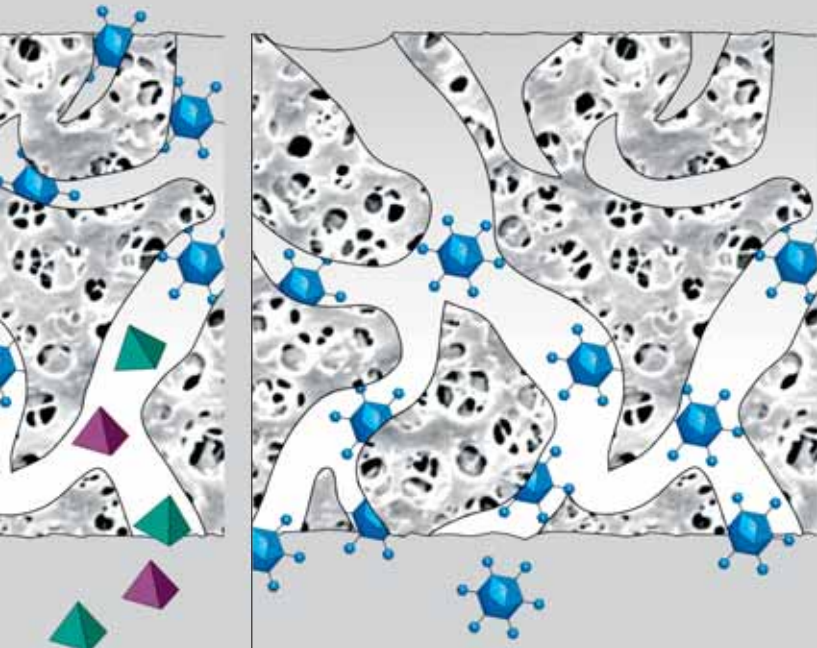
Wash away residual medium, contaminating proteins and nucleic acids with washing buffer.



Elution 15 min.

Total 100 min.

concentrate and purify adenovirus type 5 strains.



#### D. Elution

Elute purified viral particles with elution buffer.

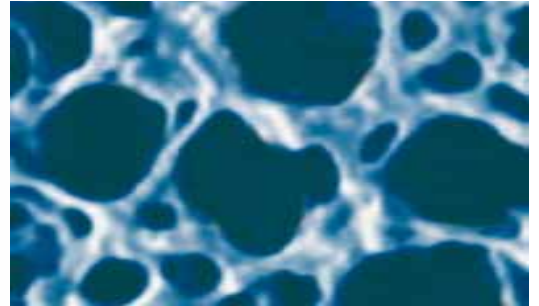
#### E. Final concentration | buffer exchange

Vivaspin 20 may be used to exchange elution buffer for appropriate physiological or storage buffer but also for virus concentration.

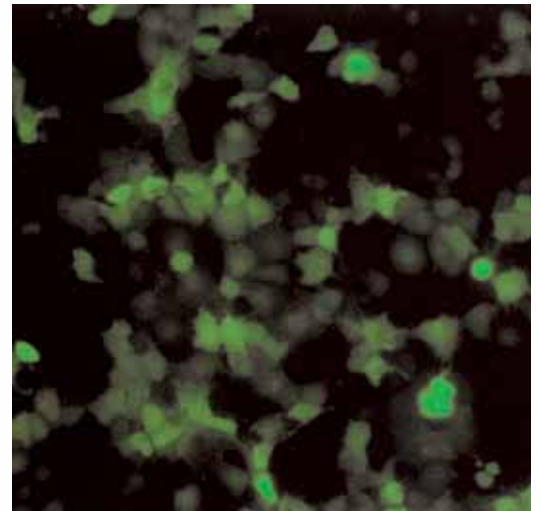
## How to use

### AdenoPACK Membrane Adsorbers

The Sartorius AG ion exchange membrane adsorber technology used in AdenoPACK encompasses the advantages of open structures of membranes with the benefits of classical chromatography.



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



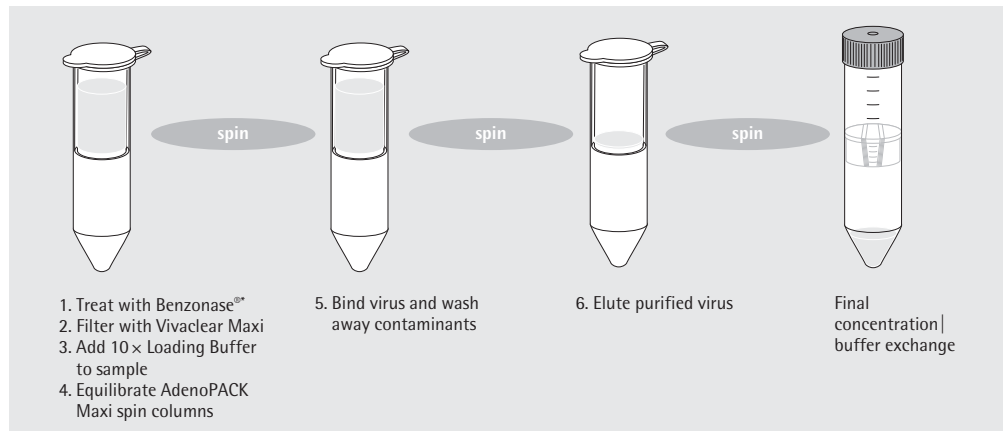
Photography: kindly provided by Dr. Lux, University Hospital and University of Applied Sciences, Mannheim. COS-1 cells infected with Ad5 GFP-constructs after purification and concentration with Vivapure AdenoPACK 100. (Fluorescent cell assay picture)

### Fluorescent cell assay show excellent virus infectivity

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 low levels of residual endotoxins allow direct use.

# Vivapure® AdenoPACK 20 – The optimal kit for construct screening

Vivapure AdenoPACK 20 is the smallest volume kit in the AdenoPACK series, purifying up to  $1 \times 10^{12}$  viral particles from 20 ml cell culture. Especially when testing new constructs, parallel and fast purifications of different adenoviruses are essential. This kit allows the rapid, simple and affordable spin column based purification of 6 different samples in parallel and bridges a gap in the CsCl density gradient method – for the first time adenovirus can efficiently be purified from less than 100 ml cell culture volume!



## Typical Performance

For a normal yielding vector,  $1 \times 15$  cm culture plate purified using this method yields up to  $1 \times 10^{12}$  viral particles.

\* Benzonase® Nuclease is manufactured by Merck KGaA, Darmstadt, Germany and is covered by US Patent 5,173,418 and EP Patent 0,229,866. Nycomed Pharma A/S (Denmark) claims worldwide patent rights to Benzonase® Nuclease, which are licensed exclusively to Merck KGaA, Darmstadt, Germany. Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.

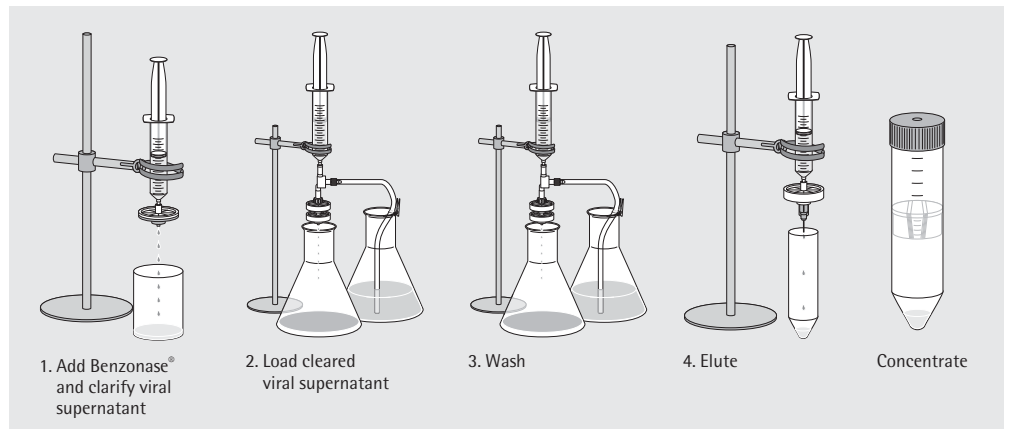
# Vivapure® AdenoPACK 100 – Fast purification of up to $1 \times 10^{13}$ viral particles

Vivapure AdenoPACK 100 is optimally suited for adenovirus purification from up to 200 ml cell culture for in vitro transfection. This flexible kit contains two AdenoPACK 100 units, which can be either used in tandem for the purification of up to 200 ml cell culture supernatant or individually for purifying virus from up to 60 ml cell culture. The purification is pursued manually with a syringe optimally attached to a retort stand. However, for even more convenience, protocols are provided for optionally running the virus purification with a peristaltic pump or with an infusion pump, in addition to detailed instructions for a manual operation supplied with the Kit. The accessories needed for the operation with a pump are supplied as individual products.



## Typical Performance

For a normal yielding vector,  $10 \times 15$  cm culture plate purified using this method yields up to  $1 \times 10^{13}$  viral particles.



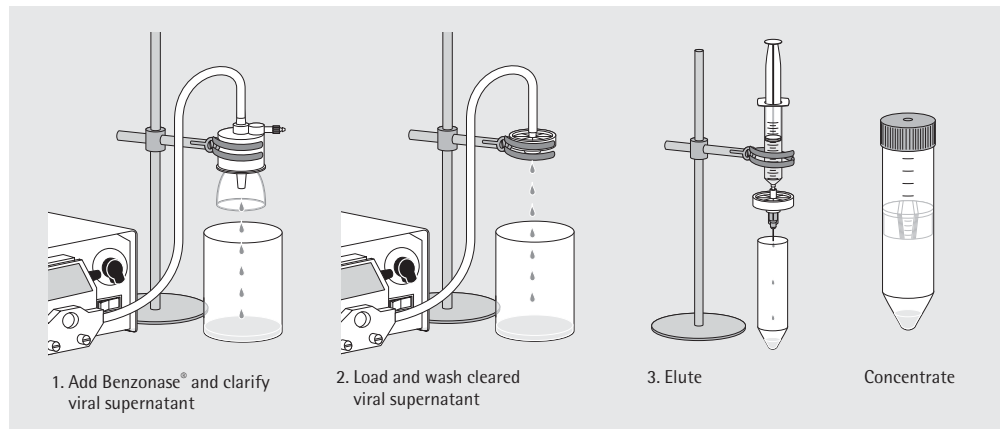
# Vivapure® AdenoPACK 500 – Pump driven Kit for larger volumes

Vivapure AdenoPACK 500 is the direct upscale kit to the AdenoPACK 100, for adenovirus purification. In only 2 hours up to  $3 \times 10^{13}$  adenovirus particles are purified and concentrated from 500 ml cell culture. This completely ready-to-use kit is conveniently operated by a laboratory pump, offering optimal flow control and minimal hands-on time. This easy to use product replaces lengthy and inefficient cesium chloride density gradient methods.



## Typical Performance

For a normal yielding vector,  $25 \times 15$  cm culture plate purified using this method yields up to  $3 \times 10^{13}$  viral particles.





# Technical Data

## Vivapure AdenoPACK 20

### Kit Specifications

Sample size	20 ml of cell culture
Number of purifications	6 x 20 ml
Virus particles (VP) per ml	Typically up to $1 \times 10^{11} - 10^{12}$
VP/IU	50 – 100
Processing time	Typically 1 hour
Endotoxin level	< 0.025 EU/ml

## Vivapure AdenoPACK 100

### Kit Specifications

Sample size	20- 200 ml of cell culture
Number of purifications	2 x 20-60 ml 1 x 200 ml
Virus particles (VP) per ml	Typically up to $1 \times 10^{13}$
VP/IU	20 – 50
Processing time	Typically 2 hours
Endotoxin level	< 0.025 EU/ml

## Vivapure AdenoPACK 500

### Kit Specifications

Sample size	500 ml of cell culture
Number of purifications	1 x 500 ml
Virus particles (VP) per ml	Typically up to $3 \times 10^{13}$
VP/IU	20 – 50
Processing time	Typically 2 hours
Endotoxin level	< 0.025 EU/ml

# Ordering Information

## Vivapure AdenoPACK 20 contents and ordering Information

Vivapure AdenoPACK 20	VS-AVPQ020
AdenoPACK Maxi spin columns	6
Vivaclear Maxi 0.45 µm PES	6
Empty 50 ml tubes	6
Loading Buffer (10×)	25 ml
Washing Buffer (10×)	30 ml
Elution Buffer	20 ml
Benzonase® (12.5 U/µl)	120 µl
Vivaspin 20, 100 kDa MWCO	6
Technical data sheet	2

## Vivapure AdenoPACK 100 contents and ordering Information

Vivapure AdenoPACK 100	VS-AVPQ101
AdenoPACK 100 RT*	VS-AVPQ102
AdenoPACK 100 units	2
Minisart Plus	4
20 ml Syringe	4
Tubing set and one way vale	1
10 ml syringe (elution)	2
Loading Buffer (10×)	1 × 25 ml
Washing Buffer	1 × 120 ml
Elution Buffer	1 × 20 ml
Elution Tip (Red)	2
Benzonase® 12.5 U/µl	200 µl
Vivaspin 20 concentrator	4
Instructions	1 each for Kit and Vivaspin

## AdenoPACK 100 Accessories

VS-AVPA001	Pump tubing set for Vivapure AdenoPACK 100
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## Vivapure AdenoPACK 500 contents and ordering Information

AdenoPACK 500	VS-AVPQ501
AdenoPACK 500 Membrane Adsorber	1
Sartopore 2 150	1
Tubing set and one way valve	2
10 ml syringe	1
Loading Buffer (10×)	60 ml
Washing Buffer (10×)	30 ml
Elution Buffer (1×)	20 ml
Benzonase® 12.5 U/µl	500 µl
Vivaspin 20 concentrator	2
Instructions	1 each for Kit and Vivaspin

\* AdenoPACK 100 RT does not contain Benzonase®

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