

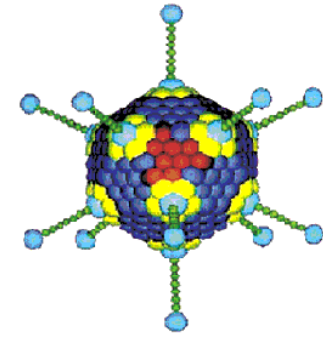
# Vivapure® AdenoPACK™ Set

## Setovi za purifikaciju & koncentraciju adenovirusa

### **Vivapure® AdenoPACK Set**

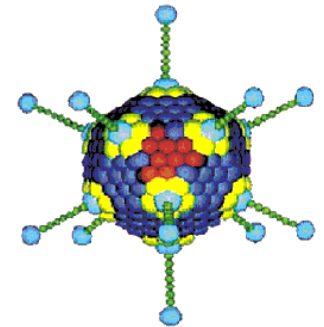
IEX Set adsorptivnih membrana

Za brzo i efikasno pročišćavanje i koncentraciju adenovirusa 5



## Uvod

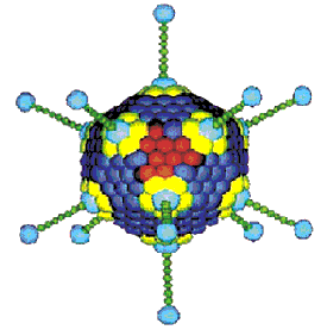
- 6 je glavnih skupina adenovirusa (A–F) sa oko 50 serotipova
- Ad5 je najčešći adenovirus korišten kao genski vektor
- Ad5 se može replicirati samo u HEK293 stanicama
- Genomom adenovirusa se može jednostavno manipulirati
- Adenovirusi mogu inficirati najraznovrsnije tipove stanica i tkiva



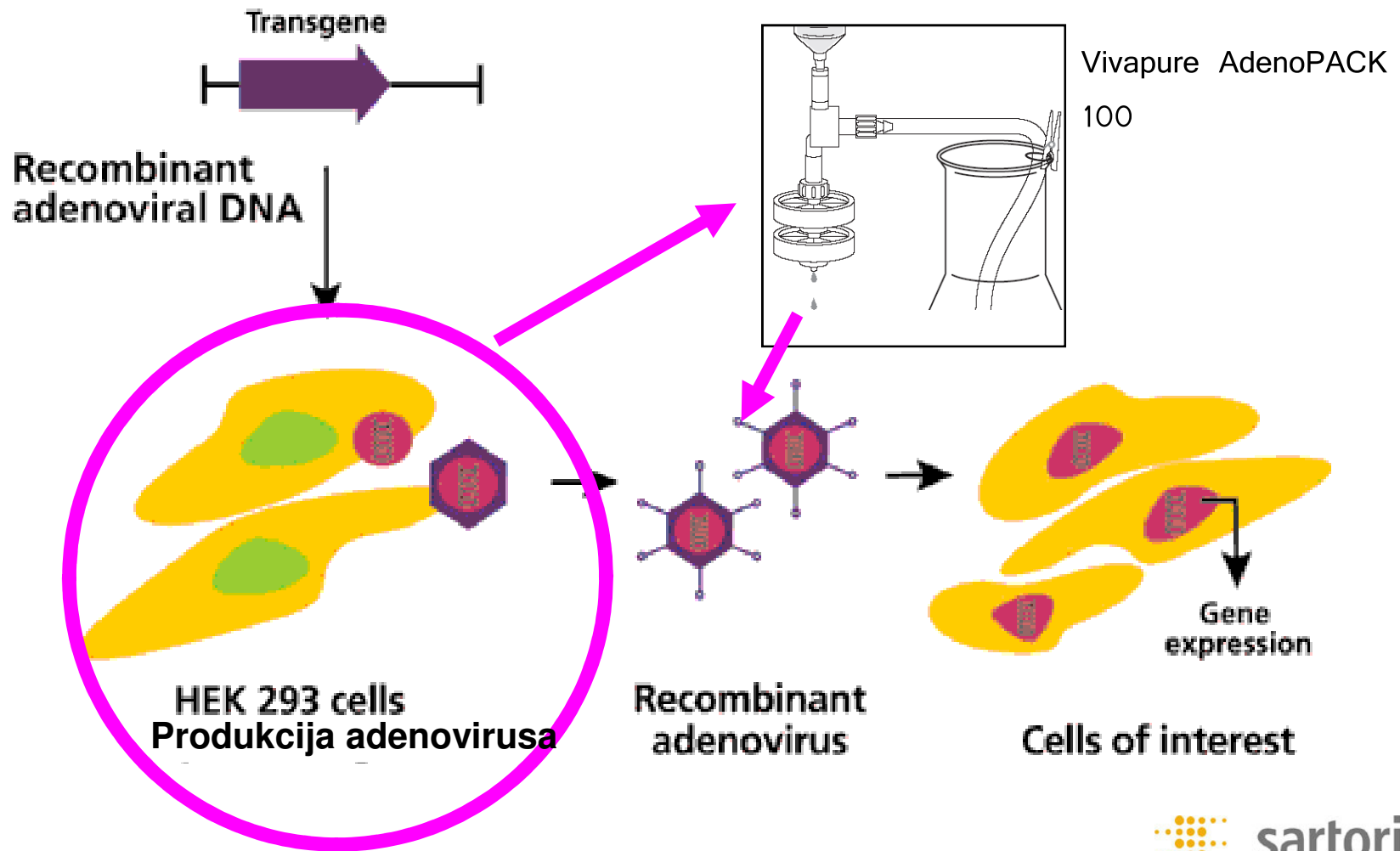
## Uvod

Primjene adenovirusa: Genski vektori za

- onkogenezu
- transkripcijsku regulaciju
- istraživanje genske terapije
- proizvodnju proteina sisavaca



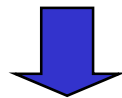
# Biologija adenovirusa



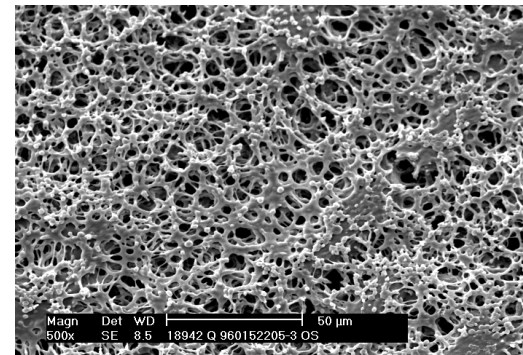
## Vivapure® AdenoPACK: Princip

Set se sastoji od Sartorius IEX adsorberskih jedinica: AdenoPACK filteri za špricu (Sartobind 15) – za hvatanje i purifikaciju virusa.

**Tradicionalna IEX kromatografija**  
+  
**Matrix membranskog adsorbera**



**Brza i efikasna purifikacija**



**IEX:** Vežanje virusa u **puferu s niskom količinom soli** (ispiranje uzorka je **vrlo važno**), ispiranje i izdvajanje sa **puferom s velikom količinom soli**

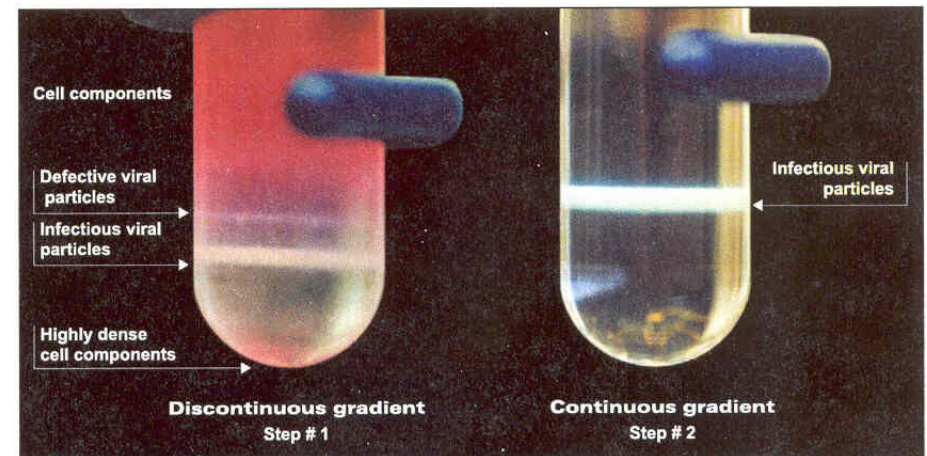
## Metode purifikacije adenovirusa

### Najčešće

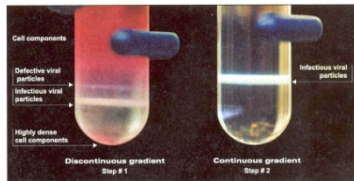
Ultracentrifugiranje sa  
CsCl – gradijentom

### Nedostaci

- Potrebna ultracentrifuga
- Dugotrajna procedura (2-3 dana)



## Purifikacija adenovirusa: Druge metode



CsCl gradient vs **Vivapure AdenoPACK**



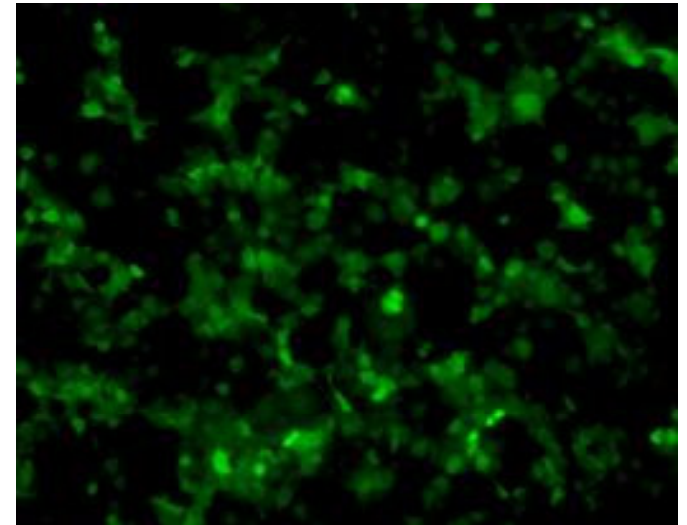
Vivapure AdenoPACK prednosti:

- 10-20 x brže – 2 sata vs. 36 sata !
- 2x veći titar virusa korištenjem istog volumena stanica
- netoksično: nije potrebna dijaliza za oslobađanje uzorka iz citotoksičnog CsCl

## Vivapure® AdenoPACK

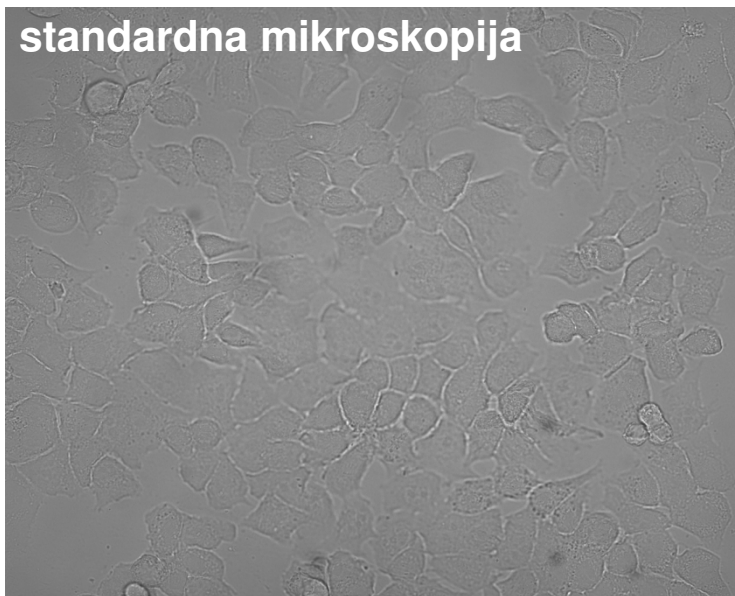
### Adeno Purification And Concentration Kit

1. **Velika čistoća** virusnih koncentrata
2. **Koncentracija** virusa (do  $3 \times 10^{13}$  VP/ml) sa vrlo velikom stopom infekcije
3. **Dobar VP/IU odnos** (ukupne čestice vs infektivne virusne čestice) : < 50

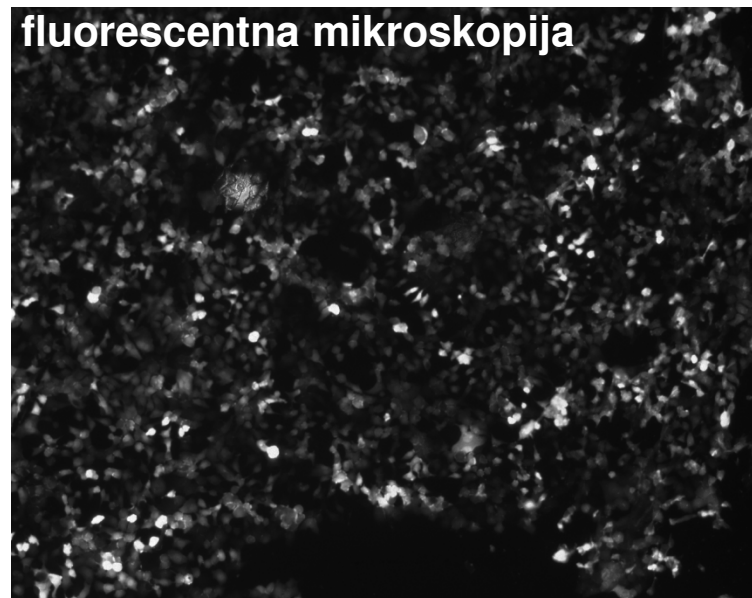


## Vivapure® AdenoPACK

standardna mikroskopija



fluorescentna mikroskopija



Djelotvorna transfekcija HeLa stanica eluatom Adenovirusa-GFP

## Vivapure AdenoPACK – Purifikacija i koncentracija adenovirusa

Setovi membranskih adsorbera za brzu i efikasnu purifikaciju i koncentraciju adenovirusa 5



### AdenoPACK 20

$1 \times 10^{11-12}$  Adenovirus iz  
20 ml kulture stanica



### AdenoPACK 100

$1 \times 10^{13}$  Adenovirus iz  
200 ml kulture stanica

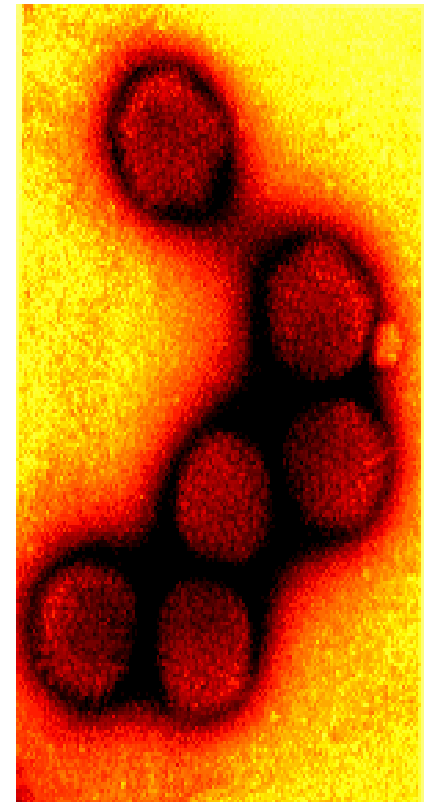


### AdenoPACK 500

$3 \times 10^{13}$  Adenovirus iz  
500 ml kulture stanica

## Vivapure® AdenoPACK: Proces purifikacije

- Infekcija (HEK 293 stanice)
- Harvest infected cells (cytophatic effects are visible)
- Cell Lysis (to release virus particles) 3 freeze/thaw cycles
- Lysate dilution 1:1 (salt and pH conditions)
- Digestion with benzonase (30 min at 37°C)
- Lysate filtration with supplied filter
- Lysate load onto AdenoPACK units
- Bound virus wash to remove impurities
- Elution of purified virus particles with high-salt buffer
- Concentrate/buffer exchange eluate with Vivaspin 20



## Vivapure AdenoPACK 20 – Kit Contents

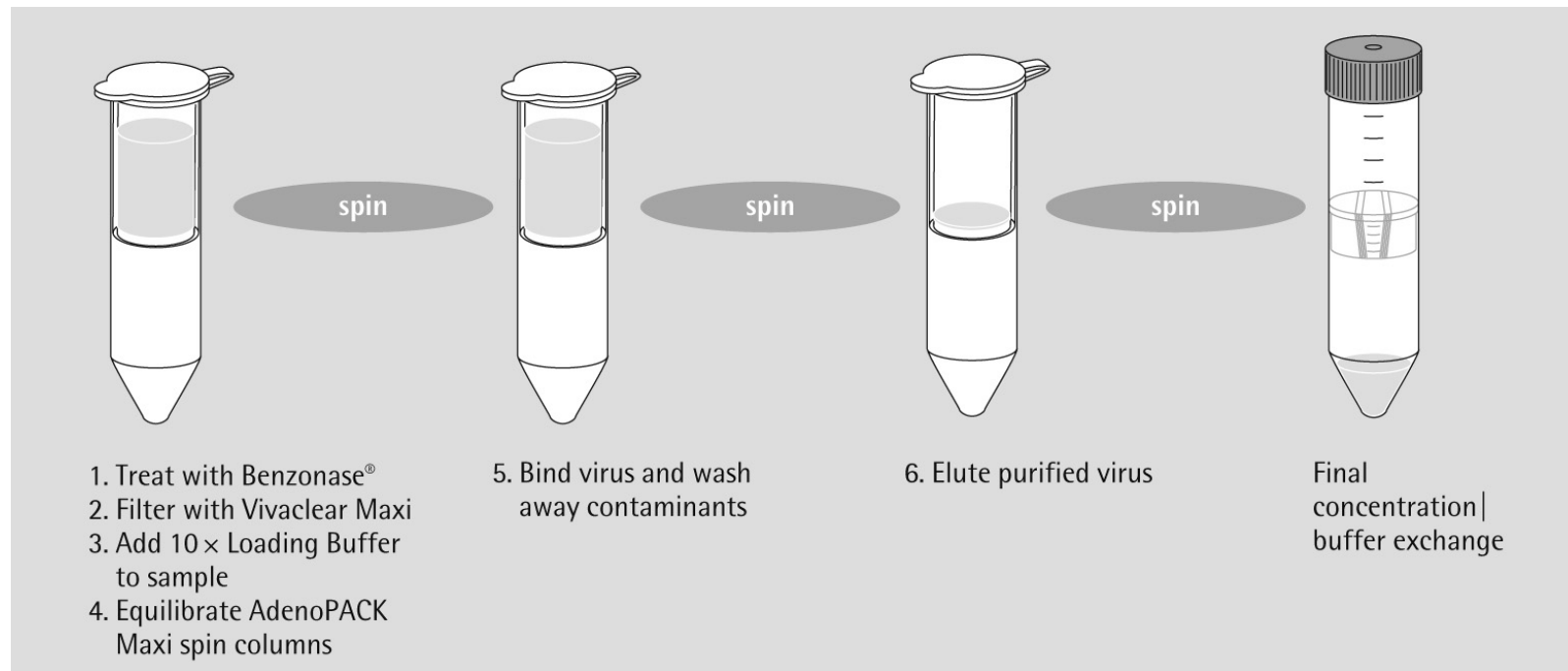
- 6 x Vivapure AdenoPACK spin columns (Vivapure Maxi Q)
- 6 x Vivaclear Maxi 0.45 mm
- 6 x Empty 50 ml tubes
- 6 x Vivaspin 20 (100 kDa)
- 120 µl Benzonase (12.5 U/ml)
- Tech data sheets (AdenoPACK, VS20)



### Supplied Buffers:

- 10x Loading Buffer (25 ml)
- 10 x Washing Buffer (30 ml)
- Elution Buffer (20 ml)

## Vivapure AdenoPACK 20 – Handling Overview



Spin column based – up to 20 ml for  $1 \times 10^{11-12}$  VPs

## Vivapure® AdenoPACK 100: Kit Contents

- 2 x AdenoPACK syringe filters  
(Sartobind 15 Q)
- 4 x Minisart Plus 0.45 mm
- 2 x 50-ml Syringes
- 4 x 10-ml Syringes
- 2 x Elution tips
- 2 x Valves/tubing
- 4 x Vivaspin 20 (100 kDa)
- 200 µl Benzonase (12.5 U/ml)
- Tech data sheets (AdenoPACK, VS20)

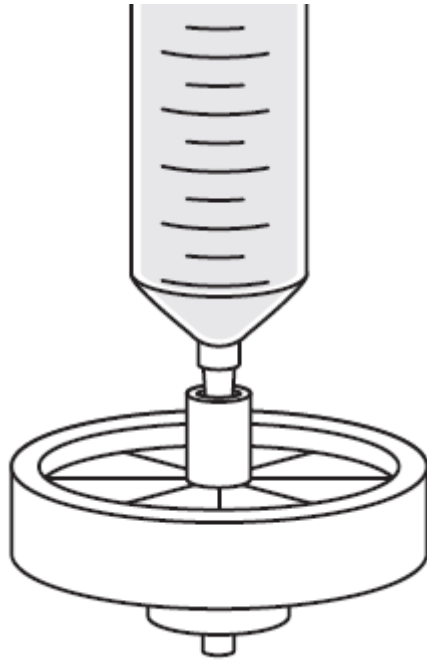


### Supplied Buffers:

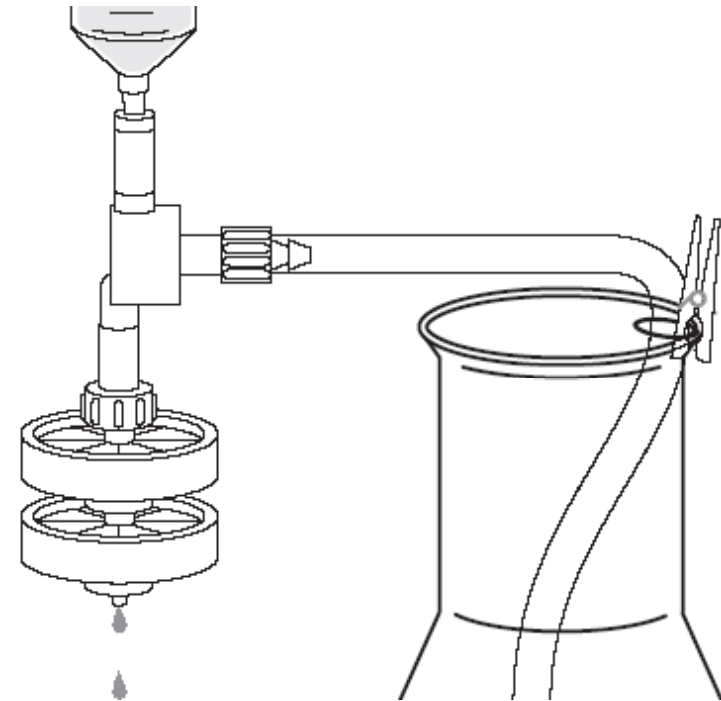
- 10x Loading Buffer (10 ml)
- Washing Buffer (120 ml)
- Elution Buffer (20 ml)

Price: 295 €

## Vivapure® AdenoPACK 100

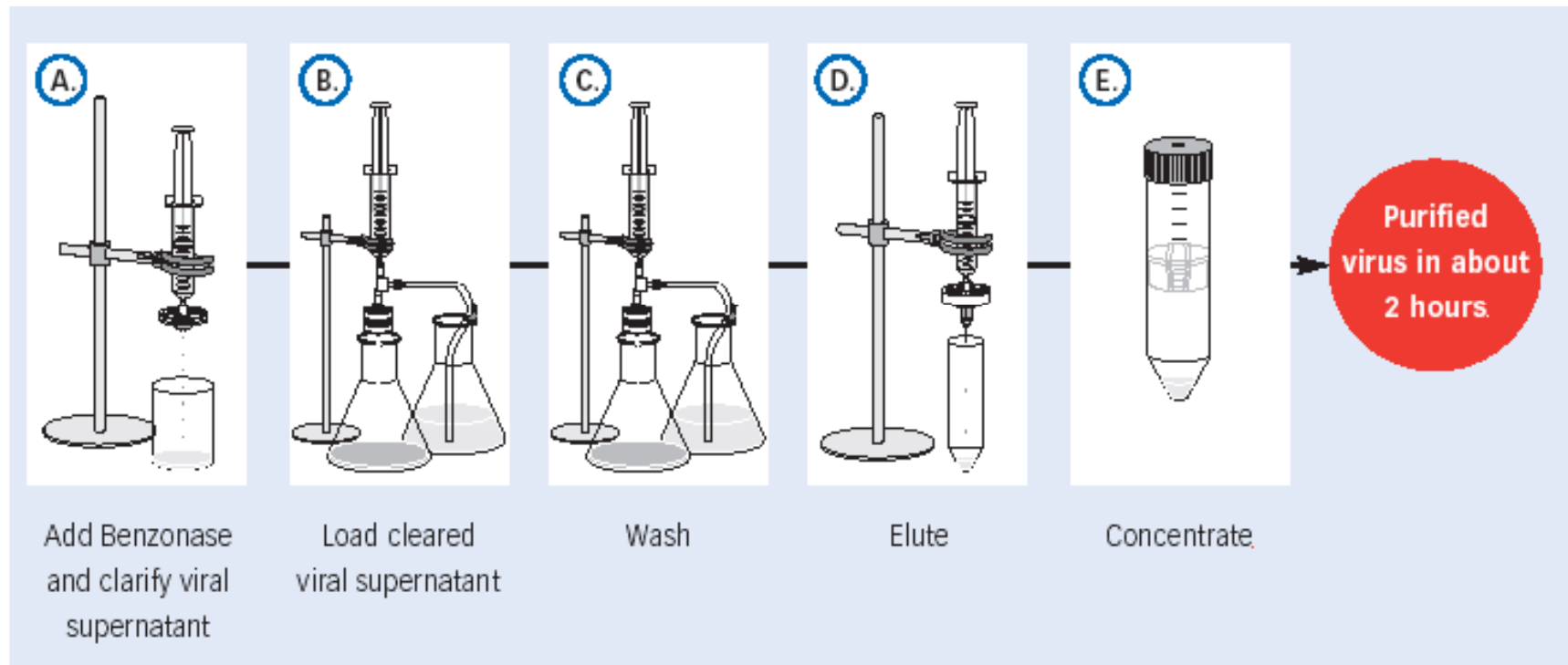


1x AdenoPACK unit for 20-60 ml cell culture



2x AdenoPACK unites in tandem for 60-200 ml cell culture

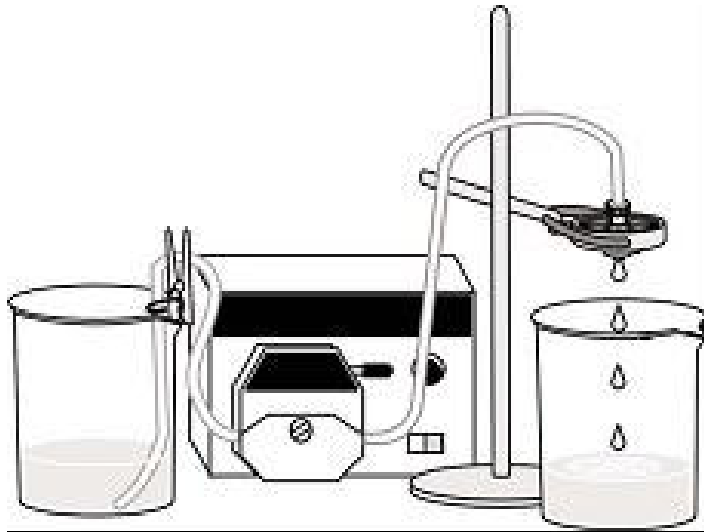
## Vivapure® AdenoPACK – Handling Overview



Syringe filter based – up to 200 ml for  $1 \times 10^{13}$  VPs

## Vivapure® AdenoPACK – Pump Operation Protocols Available

- For more convenience, a pump protocol is available on the web site for operating the AdenoPACK 100 with a pump
- Interesting for customers pursuing many preps
- Tubing and connectors need to be ordered - VS-AVPA001



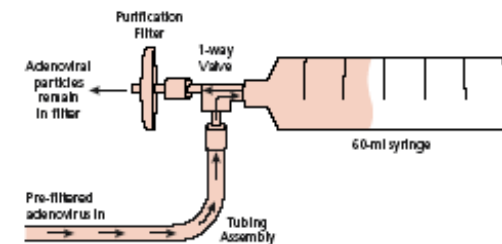
## Adenovirus Purification: Competition

Other membrane adsorber-based kits

- AdenoPURE (Puresyn Inc.)
- Adeno-X (Takara-Clontech)

Vivapure AdenoPACK advantages

- Complete kit: all included, even ultrafiltration and clarification devices
- Up-scalable to industrial virus purification: Unique product in the market
- We supply the membrane adsorber units to competitors as OEM



## AdenoPACK 500 - Kit Contents

- 1 x AdenoPACK syringe filters  
(Sartobind 75 Q)
- 1 x Sartopore 2 150
- 1 x 10-ml Syringes
- 1 x Valves/tubing
- 2 x Vivaspin 20 (100 kDa)
- 500 µl Benzonase (12.5 U/ml)
- Tech data sheets (AdenoPACK, VS20)

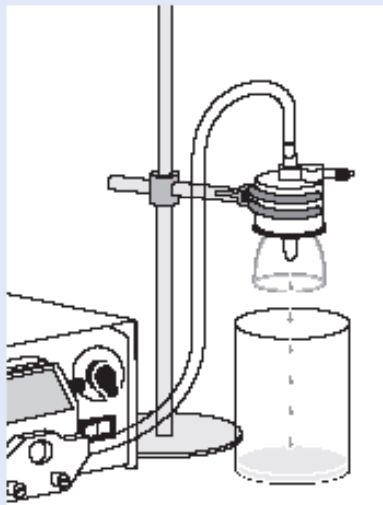


### Supplied Buffers:

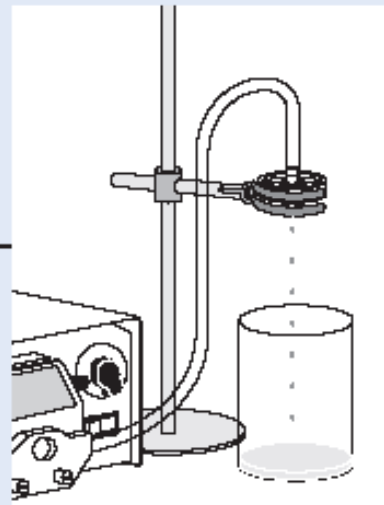
- 10x Loading Buffer (60 ml)
- 10 x Washing Buffer (30 ml)
- Elution Buffer (20 ml)

## AdenoPACK 500 - Pump Driven Kit for more Convenience

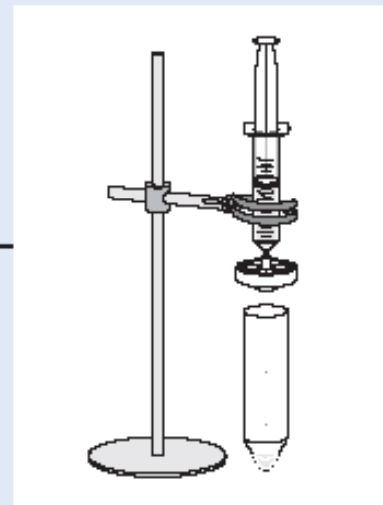
### Handling Overview - Purified virus in about 2 hours



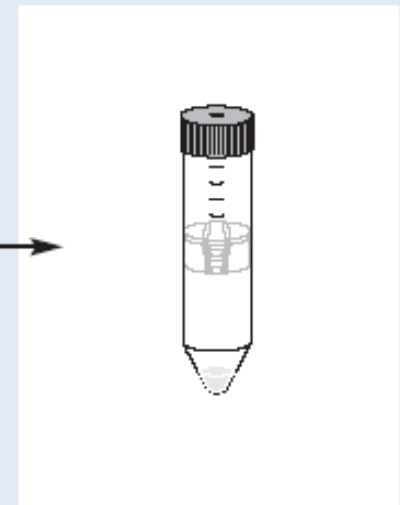
Add Benzonase® and clarify viral supernatant



Load and wash cleared viral supernatant



Elute



Concentrate

Pump driven – up to 500 ml for  $3 \times 10^{13}$  VPs

## Kit specifications

Kit specifications	
Sample size	Up to 500 ml of Adenovirus supernatants
Virus particles (VP)	Typically up to $1 - 3 \times 10^{13}$
VP/IU	20 - 50
Processing time	Typically 2 hours
Endotoxin level	<0.025 EU/ml



## Vivapure® AdenoPACK – Features and Benefits

- Complete kit → Convenient, ready-to-use
- Membrane adsorber technology → Fast and efficient virus purification
- Disposable format → Safer working with infectious material
- Mild purification procedure → High virus infectivity
- Small elution volumes → Highly concentrated virus solution
- Flexible Protocols → For 20-200 ml cell lysate

## Who and where are our customers?

- Biomedical research labs
- Vector core labs
- Biotech and Biopharm research labs
- University hospitals

Working on:

- In vitro transfection
- Primary cell lines (cardiomyocytes, liver, kidney)
- Gene delivery systems
- Gene therapy vaccines (research phase)



## How to target customers:

- Target CsCl customers on speed and convenience
- Target Takara customers on performance:

They pay for the 100 ml Kit 295€ for  $3,7 \times 10^{11}$  particles

for the 5 x 100 ml Kit 185€ for  $5 \times 3,7 \times 10^{11}$  particles

We offer: up to  $10^{13}$  particles for 295€ - more cost effective!!!

- Target Puresyn customers on completeness of our Kit

We offer: Vivaspin 20 for concentration and prefilters

Same price for virus purification – more cost effective



## Qualifying Questions (I)

### **All potential customers will be working with Adenovirus Type 5**

#### **How do you purify you adenovirus?**

- If the answer is: **CsCl gradient**
- Our kits purify up to up to  $3 \times 10^{13}$  VP from up to 500 ml infected cell culture in 1-2 hours!
- Faster and more efficient: in less time you need less serum (expensive), less plates, less cells, less media, less space to purify the same amount of virus that would normally require 40 plates and much longer time.
- Virus cannot be purified from 20 ml cell culture with CsCl gradients – however easily and quickly with AdenoPACK 20!

