SARTORIUS

Product Datasheet

Vivapure® Adenopack and Lentiselect

Rapid Viral Vector Purification, as Simple as Filtration

Benefits

- Easy virus purification in only 1 - 6 hours with minimal equipment requirements
- Screen multiple constructs in parallel at small-scale
- Convenient scale-up for purification from larger volumes
- Maximum titres and safety due to high purity and low endotoxin levels

Product Overview

Vivapure® Adenopack and Lentiselect are complete kits enabling the efficient purification of adenovirus type 5 and VSV-G pseudotyped lentivirus. Each kit has minimal equipment requirements, avoiding the high time and equipment cost associated with conventional CsCl gradient and ultracentrifugation techniques.

Featuring Sartobind® membrane adsorber technology, each kit makes viral vector purification as simple as filtration, while ensuring high titres and levels of purity for maximum infectivity and cell viability.

Viral Vectors

Recombinant viral vectors are the preferred method for a wide range of gene delivery applications. In particular, adenovirus type 5 and VSV-G pseudotyped lentivirus are two examples which are frequently used for *in vitro* and *in vivo* applications.

Adenovirus vectors are versatile tools in research and therapeutic applications, where they are used for gene transfer, protein expression in cell lines that have low transfection efficiency with liposomes, and RNAi delivery. After entering cells, the virus remains epichromosomal (i.e. it does not integrate into the host genome). Lentiviral vectors are frequently used in gene transfer studies, due to their capability for integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens their target cell range. Lentiviral vectors have been shown to deliver genes into cell types such as neurons, lymphocytes and macrophages, which other retroviral vectors cannot be used for. The lentivirus vector is increasingly used to integrate siRNA efficiently in a wide variety of cell lines and primary cells, both *in vitro* and *in vivo*.

Product Information

The Sartobind® ion exchange membrane adsorber technology used in Vivapure® Adenopack and Lentiselect kits is unique in its capability to efficiently and rapidly capture and recover large virus particles. When compared to chromatography media, membrane adsorbers feature significantly larger pore sizes of > 3 µm, allowing unrestricted access and recovery of virus from the functionalized surface.

Convective flow through the centrifugal and syringe filter devices provides high speed separations not possible with conventional chromatography, cesium chloride density gradients or ultracentrifugation methods. The porous matrices, high capacities, low differential pressures, high flow rates and low non-specific adsorption of Sartorius membrane adsorbers contribute to superior performance in small scale virus purification.

In addition, for convenient, GMP compliant scale-up, Sartobind® capsules and cassettes offer high performance purification from larger batch volumes, while benefiting from as much as 10-fold reductions in process time.

Adenovirus Purification

Adenopack 20, 100 and 500
Adenovirus purification and concentration kits offer researchers who need to recover up to 3×10^{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 straightforward kits replace time-consuming and labor-intensive 48 hour CsCl density gradients.

Three kit sizes cater for the purification and concentration of adenovirus type 5 from 20 to 500 mL cell cultures, resulting in titres of 1×10^{11} to 3×10^{13} viral particles. For ultimate convenience, each kit utilizes the most efficient handling methods, suited to the sample volumes being processed. To this end, Adenopack 20 kits feature centrifugal devices, while Adenopack 100 and 500 utilize syringe and pump-driven filters, respectively.

With Adenopack, adenovirus purification is completed over ten times faster than with CsCl density gradients. Furthermore, instead of using only the viral pellet, adenovirus is purified from the entire cell culture, leading to higher virus titres. The three kit offerings cater for initial construct screening, right up to large-scale purifications and with low endotoxin levels of < 0.025 EU/mL, you can expect to benefit from high cell viabilities and infection rates.

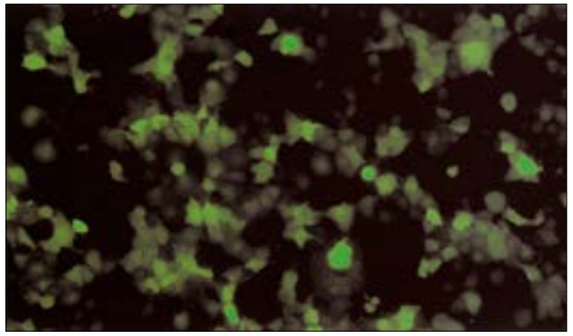
Performance Characteristics for Adenopack

Purification of Ad5-GFP Constructs

Purification Method	Sample Volume	Process Time	Eluate	Recovery**	Viral Particles
Adenopack 20	20 mL	1 hr	1 mL	65 – 70%	$1 \times 10^{11\text{-}12}$
Adenopack 100	60 mL	1 – 2 hr	1 mL	65%	$1 – 3 \times 10^{12}$
Adenopack 100	200 mL	2 hr	1 mL	80%	1×10^{13}
Adenopack 500	500 mL	2 hr	1 mL	80%	$1 – 3 \times 10^{13}$
CsCl density gradient	500 mL	24 – 48 hr	1 – 2 mL*	60 – 70%	$1 \times 10^{11\text{-}12}$

* After dialysis

** Before buffer exchange

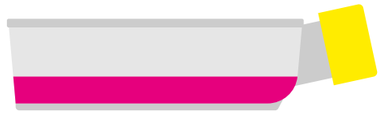


Photography: kindly provided by Dr. Lux, University Hospital and University of Applied Sciences, Mannheim. COS-1 cells infected with Ad5 GFP-constructs after purification and concentration with Vivapure® Adenopack 100.

Purified Adenovirus Infectivity by Fluorescence Microscopy
Recombinant Adenovirus-GFP, purified with Adenopack 100 was used to infect HeLa cells. Since the construct contains a GFP gene, transfected cells are easily visualized by fluorescence microscopy. Cell viability and infection rate determined by this method indicate high virus purity and low endotoxin levels in the purified virus, confirming suitability for direct use in cell culture.

Adenopack Principle of Operation

Sample Preparation (45 min)
Infect HEK 293 cells with adenovirus stock until most show cytopathic effects. Harvest cells and lyze by freeze | thaw cycles. Remove cell debris by centrifugation. Treat the supernatant with Benzonase®, filter and dilute the sample with 10× loading buffer.

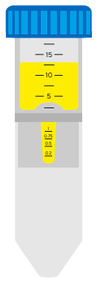


Sample Loading (30 min)
Apply the prepared supernatant through the Adenopack unit. When using Adenopack 100, use a single purification unit for up to 60 mL of cell culture supernatant or use both units in tandem for up to 200 mL.

Washing (10 min)
Remove residual cell culture medium, and contaminating proteins and nucleic acids with washing buffer.

Elution (15 min)
Elute purified viral particles with elution buffer.

Buffer Exchange | Concentration
The included Vivaspin® 20 devices may be used to exchange the purified virus into an appropriate physiological or storage buffer and also for virus concentration.



Lentivirus Purification

Lentiselect 40, 500 and 100
Lentivirus purification and concentration kits offer researchers who need to recover up to 4 – 5 × 10^9/mL purified recombinant lentivirus particles for *in vitro* transfection a fast, safe and easy to use solution. Similar to the Adenopack kits, they include all reagents and devices necessary for purification and concentration of VSV-G pseudotyped lentivirus from HEK293 cell cultures. These straightforward kits replace time-consuming ultracentrifugation protocols, which typically take a full day for large sample volumes, reducing the purification time to only a few hours.

Three kit sizes are available for the purification and concentration of VSV-G pseudotyped lentivirus from 40 mL to 1 L cell cultures, yielding high titres of 8 × 10^8 to 1 × 10^{10} infective particles. For each sample volume, the most convenient handling method is offered. To this end, 40 mL samples are processed manually with Lentiselect 40, while Lentiselect 500 and 1000 are pump driven kits.

Lentiselect kits make lentivirus purification as easy as filtration. With no need for expensive ultracentrifuges, purifications are completed in only 1 - 6 hours. Furthermore, the chromatographic membrane adsorption technique avoids crude and variable viral pellets, leading to higher virus purity, and with low endotoxin levels of < 0.025 EU/mL, you can expect to benefit from high cell viabilities and infection rates.

Performance Characteristics for Lentiselect

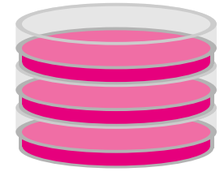
Purification of VSV-G Pseudotyped Lentivirus Constructs

Kit	Sample Volume	Process Time	Eluate	Recovery	Viral Particles
Lentiselect 40	40 mL	45 min	0.2 mL*	50%	8 × 10^8
Lentiselect 500	500 mL	3 hr	1 mL*	35%	2 – 5 × 10^9
Lentiselect 1000	1,000 mL	6 hr	2 mL*	35%	1 × 10^{10}
Ultracentrifugation	500 mL	10 - 11 hr	0.5 mL	25%	3 × 10^9

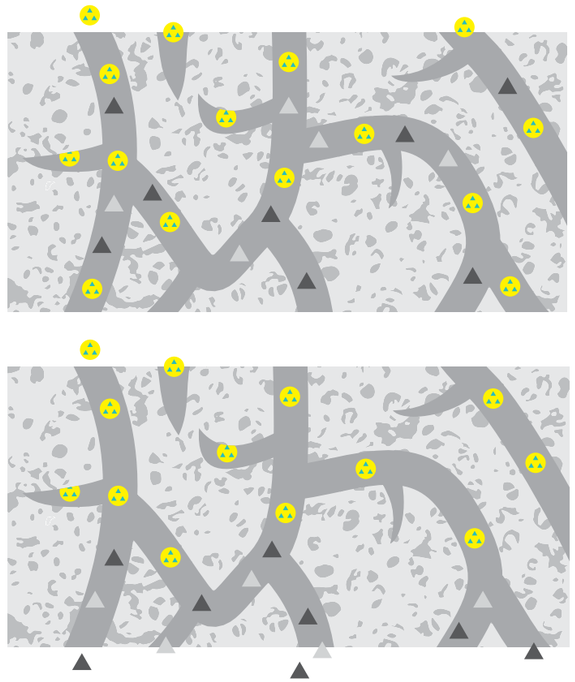
*After desalting | buffer exchange

Lentiselect Principle of Operation

Sample Preparation (5 min)
Culture HEK 293 cells, and co-transfect with packaging plasmid mix and expression construct. Equilibrate Lentiselect purification unit using 40 mL of 1 × loading buffer.



Sample Loading (20 min)
Place end of the feed tube into one 15 cm cell culture plate and aspirate the supernatant. Repeat this step with a second plate, taking care not to exceed the maximum 40 mL sample volume. Pass the sample slowly through the Lentiselect unit.



Washing (5 min)
Remove residual cell culture medium, and contaminating proteins and nucleic acids with washing buffer.

Elution (15 min)
Elute purified viral particles with elution buffer.

Buffer Exchange | Concentration
The included Vivaspin® 20 devices may be used to exchange the purified virus into an appropriate physiological or storage buffer and also for virus concentration.

Technical Specifications

Vivapure® Adenopack Kits

	Adenopack 20	Adenopack 100	Adenopack 500
Number of purifications	6 × 20 mL	2 × 20 - 60 mL or 1 × 200 mL	1 × 500 mL
Typical virus titre	1×10^{11} - 1×10^{12} VP/mL	1×10^{13} VP/mL	3×10^{13} VP/mL
Viral particle to plaque forming unit ratio (VP/PFU)	50 - 100	20 - 50	20 - 50
Typical processing time	1 hr	2 hr	2 hr
Endotoxin level	< 0.025 EU/mL	< 0.025 EU/mL	< 0.025 EU/mL

Vivapure® Lentiselect Kits

	Lentiselect 40	Lentiselect 500	Lentiselect 1000
Number of purifications	4 × 40 mL	1 × 500 mL	1 × 1,000 mL
Typical number of infective particles	3×10^{9} IP/mL	2 - 5 × 10^{9} IP/mL	4 - 5 × 10^{9} IP/mL
Final sample volume	0.2 mL	1 mL	2 mL
Viral particle to plaque forming unit ratio (VP/PFU)	5 - 15	20 - 50	20 - 50
Typical processing time	45 min	3 hr	6 hr
Endotoxin level	< 0.025 EU/mL	< 0.025 EU/mL	< 0.025 EU/mL

Ordering Information

Vivapure® Adenopack	Order No.
Vivapure® Adenopack 20, for 6 × 20 mL purifications, with Benzonase®	VS-AVPQ020
Vivapure® Adenopack 20 RT, for 6 × 20 mL purifications	VS-AVPQ022
Vivapure® Adenopack 100, for 2 × 60 mL or 1 × 200 mL purifications, with Benzonase®	VS-AVPQ101
Vivapure® Adenopack 100 RT, for 2 × 60 mL or 1 × 200 mL purifications	VS-AVPQ102
Vivapure® Adenopack 500, for 1 × 500 mL purification, with Benzonase®	VS-AVPQ501
Vivapure® Adenopack 500 RT, for 1 × 500 mL purification	VS-AVPQ502

Vivapure® Adenopack Accessories	Order No.
Pump tubing set for Adenopack 100	VS-AVPA001

Vivapure® Lentiselect	Order No.
Vivapure® Lentiselect 40, for 4 × 40 mL purifications	VS-LVPQ040
Vivapure® Lentiselect 500, 1 × 500 mL purification	VS-LVPQ500
Vivapure® Lentiselect 1000, for 1 × 1 L purification	VS-LVPQ1000

Vivapure® Lentiselect Accessories	Order No.
Peristaltic pump	VF-APD0001-1
Peristaltic pump head for 1.6 mm WT tubing	VF-APH0001-1

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