Viral Purification Handbook

Add-N-Pure™	(A902, A905 A910)
PuRetro™	(G171, G172, G173)
Buffer Exchange	(G130)

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Biosafety

Recombinant adenoviral, retroviral, and lentiviral vectors have been designated as Level II biological agents by the Centers for Disease Control of USA and other regulatory bodies of different jurisdictions worldwide. Therefore, all published BL-2 guidelines with proper waste decontamination should be strictly followed. In addition, exercise extra caution when creating recombinant virus carrying potentially harmful or toxic genes (e.g. activated oncogenes).

For more information about the BL-2 guidelines and handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories", 4th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded from the Web at the following address:

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

It is also important to consult with the health and safety officers for guidelines at your institution regarding the use of recombinant viruses. **Always follow standard microbiological practices, which include:**

- Wear gloves and lab coat at all times.
- Always work with pseudoviral particles in a Class II culture facility. All virus purification work should be done in a biosafety cabinet (excluding centrifugation and water bath steps).
- All procedures are performed carefully to minimize splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.

Follow federal, state and local regulations for disposal of potentially biohazardous wastes and handle all recombinant viruses in compliance with established institutional guidelines. Since safety requirements for use and handling of recombinant viruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.

Protocol at a Glance

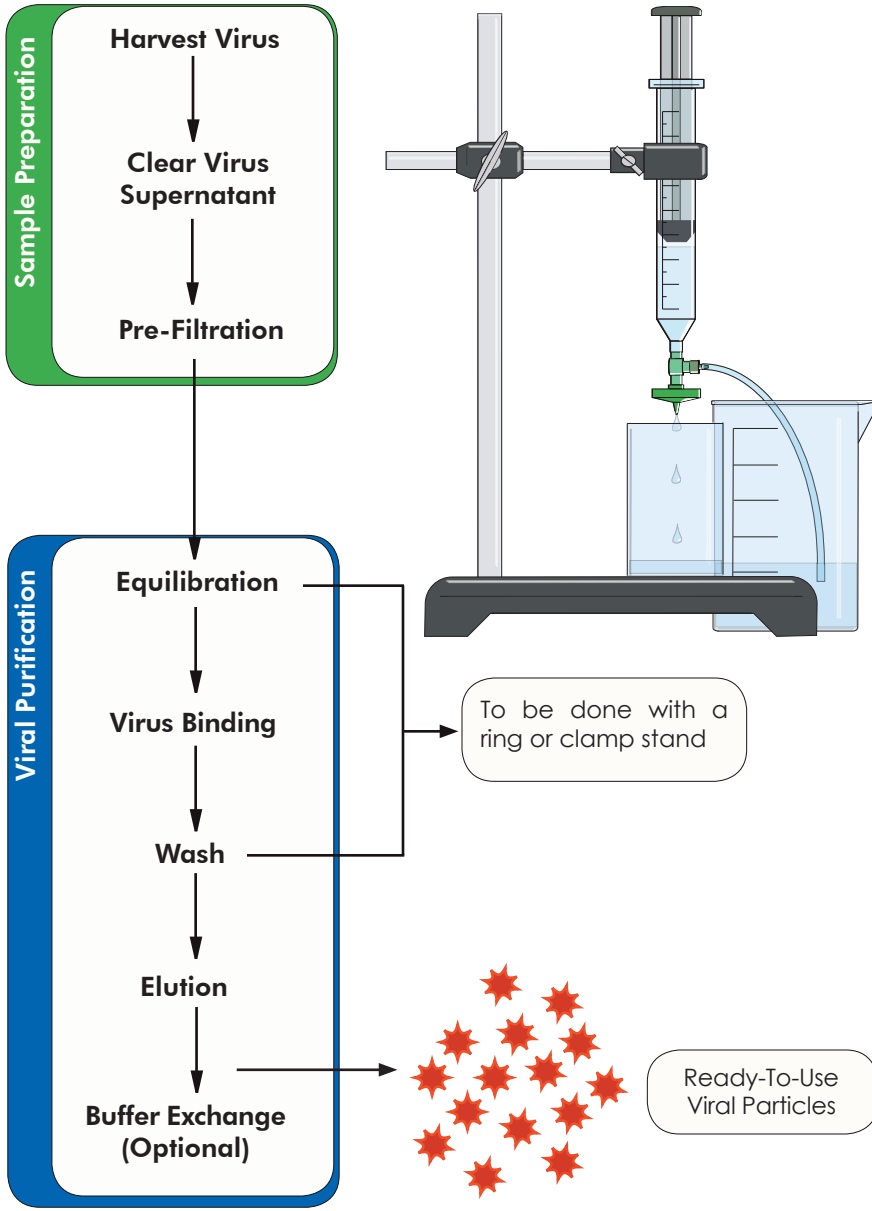

Figure 1: *Experimental Flow Chart.*

Add-N-Pure™ Adenovirus Purification

Recombinant adenovirus is the most efficient viral system developed to-date with regards to gene, siRNA, and miRNA delivery for *in vitro* and *in vivo* applications. However, high titers of purified recombinant adenovirus are required for many applications, especially *in vivo* gene delivery. Traditionally, purification of recombinant adenovirus is achieved by Cesium chloride density gradient ultra-centrifugation. This process is technically demanding, time consuming, and requires the availability of ultra-centrifugation equipment. Thus, there has always been a need for the development of a simpler and more efficient adenovirus purification method to support the increasing range of adenoviral applications.

Add-N-Pure™ adenovirus purification kit is a chromatography-based system for adenovirus purification and concentration. The kit represents a significant improvement to the traditional Cesium chloride ultra-centrifugation method, specifically in terms of easy manipulation, reliability, and efficiency. In fact, the entire Add-N-Pure™ purification process takes less than 2 hours to complete, whereas the Cesium chloride method takes 48 hours to complete the same task.

The Add-N-Pure™ purification unit comes pre-assembled in the kit for immediate use. The one-way valve allows selective withdrawal and elution in the desired direction, facilitating the purification process. The adenovirus purification filter contains an ion exchange membrane that has a high affinity for adenovirus and can be efficiently eluted under our proprietary buffer conditions. Our Add-N-Pure™ system has been tested to be more reliable and consistent than traditional Cesium chloride ultracentrifugation.

The Add-N-Pure™ adenovirus purification kit can purify and concentrate recombinant adenovirus to a concentration between 10^{10}pfu/ml to 10^{13} pfu/ml, depending on the amount of viral input, whether the Mega or Giga filter is used, and whether buffer exchange is performed. Titers up to 10^{10} to 10^{12} pfu/ml can be obtained with Cat.#A902 and Cat.#A905 purification units, whereas titers of 10^{11} to 10^{13}pfu/ml are achievable with the Cat.#A910 purification unit. **However, it is important to note that the titers are significantly influenced by the original titer of the recombinant adenovirus input.** Recombinant adenovirus which expresses proteins or regulatory DNAs toxic to packaging 293 cells, will result in notably low viral titer in the original stock.

Kit Contents

Table 1. Kit Components

Component	Maxi (2X) (Cat.# A902)	Maxi (5X) (Cat.# A905)	Giga (2X) (Cat.# A910)
10X Preparation Buffer	10ml	25ml	30ml
Equilibration Buffer	10ml	25ml	20ml
Wash Buffer	80ml	2X100ml	80ml
Elution Buffer	20ml	50ml	20ml
10ml Syringe	2X	5X	2X
20ml Syringe	2X	5X	2X
Syringe Filter	4X	10X	6X
Add-N-Pure™ Purification Unit*	2X	5X	2X

* ***Note:*** *The Add-N-Pure™ has been tested for single use only.*

Additional Materials Required

General Lab Equipment

- Ring stand with clamps
- Ethanol/dry ice bath
- 37°C water bath
- Centrifuge

Fluid Collection

- 150 – 500ml beakers for flow-through waste collection
- Sterile 15ml and 50ml conical tubes

Adenovirus Production

- Tissue culture plates and flasks
- DMEM + 10% fetal bovine serum

Protocol

The Add-N-Pure™ filter is designed for single application only. Each filter included in the kit has the capacity of binding and purifying recombinant adenovirus from 5X10cm to 5X15cm dishes of packaging 293 cells with complete cytopathic effect (CPE). Overall, a maximum of 50ml viral supernatant can be processed with Cat.#A902 and A905 kits. For the Giga Add-N-Pure™ purification unit (Cat.#A910), 150ml of viral supernatant is its capacity limit.

A. Adenovirus Production

1. **To amplify adenovirus, we recommend growing 293 cells in one well of a** 6-well plate and in one 10cm dish.
2. **When cells are approximately 60% to 70% confluent in the 6-well plate,** add 100µl of the adenoviral stock at a titer of 10^6 to 10^9pfu/ml to 0.5ml of complete culture medium in a separate tube. Aspirate the culture medium from the 6-well plate and then add the diluted virus onto the 293 cells slowly without dislodging the cells. Return the plate to 37°C / 5% CO_2 incubator for 1-2 hours before adding another 1.5ml of complete medium into the well. It will take 3-6 days to observe over 90% of the cells detached from the plate (CPE).
3. **While adenovirus is being replicated in the 6-well plate, subculture the** 10cm dish to five 10cm or one 15cm dish, which can then be re-subcultured to 5X15cm dishes later on. When 293 cells reach 60% to 70% confluency in the 10cm or 15cm dishes, add 300-400µl of crude viral stock from the previous 6-well plate directly into the 10cm or 15cm culture dishes. It will take another 4-5 days before the completion of CPE.

B. Adenovirus Harvest

4. **Collect all cells and culture medium into one or more 50ml conical tubes** when CPE is complete.
5. **Freeze-thaw the cells and culture medium 3 times to release virus from** cells. Complete the freezing step using an ethanol/dry ice bath and the thawing step using a 37°C water bath.
6. **Pellet the cell debris by centrifugation at 2,000g for 10 minutes.**

Protocol

7. **Transfer the supernatant to a new, sterile conical tube.**

8. **Add 10X Preparation Buffer to the supernatant.** The 10X Preparation Buffer should be 1/9 of the supernatant volume. Mix gently and thoroughly by inverting the tube 5-6 times.

9. **Using a 20ml syringe, complete a pre-filtration step for the supernatant** with syringe filter (included in the kit). For the Giga kit, viral supernatant over 50ml should be split up between two syringe filters. Pre-filtration will decrease viscosity and improve the flow rate of the sample during virus purification steps.

   Note: *The syringe filter is a low protein-binding type to avoid loss of recombinant virus.*

C. Adenovirus Purification

10. **Clamp the syringe column of the Add-N-Pure™ Purification Unit onto** a ring/clamp stand. Place a beaker under the filter unit for waste collection. Alternatively, a syringe pump can be used for the following procedures.

11. **Equilibrate an Add-N-Pure™ Purification Unit with 5ml of Equilibration** Buffer or a Giga Add-N-Pure™ Purification Unit with 10ml of Equilibration Buffer. Place the feed tube into a 50ml conical tube containing Equilibration Buffer and pull up the syringe plunger for withdrawal of the desired amount of buffer. Dispense the buffer through the Add-N-Pure™ Purification Unit by pressing down lightly on the syringe plunger. The buffer should flow through the filter unit at a rate of 3ml/min (approximately 1 drop per second).

    Note: *Avoid dispensing air through the Add-N-Pure™ Purification Unit. The presence of air in the filter may cause uneven distribution of solutions in the membrane and alter the efficiency of adenovirus purification. Remove the filter first before dispensing the residual air from the syringe.*

12. **Load adenoviral supernatant into the Add-N-Pure™ Purification Unit.** Place the feed tube into the conical tube containing the unpurified viral supernatant and pull up the syringe plunger to withdraw. Multiple withdrawals may be required to pass all the viral supernatant through the filter. Dispense the supernatant through the Add-N-Pure™ Purification Unit at a rate of 3ml/min (approximately 1 drop per second).

    Note: *To avoid dispensing air through the filter unit in this and the following step, leave a small volume of supernatant in the syringe. This will not affect the purification process.*

Protocol

13. **Wash the Add-N-Pure™ Purification Unit with Wash Buffer.** The volume of the Wash Buffer should equal the volume of the original culture medium. However, Wash Buffer volumes over 40ml are unnecessary. Wash Buffer volumes of 40ml is enough when input viral volume is over 40ml. Place the feed tube into the 50ml conical tube containing adequate volume of Wash Buffer and pull up the syringe plunger for withdrawal. Multiple withdrawals may be required to pass all the Wash Buffer through the filter. Dispense through the Add-N-Pure™ Purification Unit at a rate of 3ml/min (approximately 1 drop per second).

D. Adenovirus Elution

14. **Remove the plunger from a sterile 10ml syringe (provided in the kit).**
15. **Remove the filter from the Add-N-Pure™ Purification Unit.** Attach the filter to the sterile 10ml syringe.
16. **Add 3ml of Elution Buffer to the syringe column.** If the Giga filter purification unit is used or the elution is to undergo buffer exchange for *in vivo* applications or higher titer concentration, up to 10ml Elution Buffer can be used to increase the elution efficiency.
17. **Replace the plunger in the syringe.**
18. **Elute and discard the first 1.0ml of Elution Buffer into a waste** container. The first 1.0ml is largely comprised of Wash Buffer and should not be kept. Leave the rest of the buffer in the syringe column.
19. **Incubate the filter at room temperature for 5 minutes.** Rest filter on top of a sterile 15ml conical tube to catch any premature elution.
20. **Elute at a rate of 1.0ml/min (approximately 1 drop every 3 seconds)** into a sterile 15ml conical tube. Use the residual air in the syringe to help expel remaining Elution Buffer from the filter. The eluted buffer contains the purified adenovirus.

E. Post-Purification Notes

21. **Filter-sterilize viral stock post-purification using a low protein-binding** syringe filter unit (provided in the kit).

    Note: *This is to be done only if Viral Stock Buffer Exchange is not performed. Otherwise, perform this step after buffer exchange (Step 12, Page 16)*

22. **The eluted adenovirus is in a salt buffer but can be used directly for** *in vitro* target cell transduction if the viral dilution is over 5X (i.e. 1.0µl of viral stock to 4µl culture medium). For higher titers of viral stock or viral dilutions less than 5X during transduction, a buffer exchange is required to make the viral stock suitable for such applications. This can be easily performed using ABM Inc.'s viral buffer exchange kit (Cat.# G130 on page 15).
23. **For higher titers, long-term storage of the virus, and proper tonicity** for *in vivo* applications, we recommend buffer exchange of the eluted adenovirus into 1X Storage Buffer using ABM Inc.'s Buffer Exchange kit (Cat.# G130).
24. **1X Storage Buffer: 2.5% glycerol (w/v), 25mM NaCl, and 10mM Tris-HCl,** pH 8.0 (Hoganson, et al., 2002).
25. **Store final viral stock aliquots at -70°C in a non-frost-free freezer.** Avoid repeated freeze-thaw cycles.

F. Determining Adenoviral Titer

There are three protocols for determining adenoviral titer:

26. **Plaque Assay (approx. 2-3 weeks in duration)**
27. **End-Point Dilution Assay (approx. 10 days in duration)**
28. **OD_{260} Assay (approx. 1 hour in duration)**

Methods 1 and 2 are biological assays; they measure the number of infectious viral particles. Method 3 is a physical assay; it measures the concentration of viral DNA and viral protein, and therefore, does not distinguish between infectious and non-infectious viral particles.

For detailed information about adenoviral titer assay, please refer to our online technical support:

http://www.abmgood.com/TechSupport/adeno-vec.php

G. Transduction of Target Cells

For detailed information about target cell transduction with recombinant adenovirus, refer to our adenoviral expression manual:

http://www.abmgood.com/viralexp/Adeno-1%20Expresion%20System.pdf

PuRetro™ Lentivirus and Retrovirus Purification

Recombinant retrovirus is a widely used vehicle for successful stable expression of any active dividing cell type (Mann et al., 1983; Miller & Buttimore 1986). Retrovirus can efficiently integrate into the host genome, giving rise to permanent and stable gene expression. During cell division, the nuclear membrane disintegrates, allowing retroviral DNA to access host genome. Because recombinant retrovirus cannot actively pass through the nuclear membrane, the transduction efficiency of target cells with recombinant retrovirus is low, especially in slow–dividing primary cells.

Recently, recombinant lentivirus, a new type of retrovirus, has been developed to transduce both dividing and non-dividing cells at a much greater efficiency than retrovirus. Furthermore, lentivirus has the ability to transduce more cell types than recombinant retrovirus, including many primary cell types. Cells that cannot be transduced by recombinant retrovirus are often receptive to recombinant lentiviral transduction. This has led to numerous and diverse applications of recombinant lentivirus for both *in vitro* and *in vivo* gene delivery.

Both recombinant retrovirus and lentivirus can be produced by transient transfection, from which viral supernatant can be collected and used for target cell transduction. The titer of viral supernatant by transient transfection is approximately 10^6 cfu (colony forming unit)/ml, which is sufficient for the generation of most stable cell lines *in vitro*. However, there are applications that require higher purity and titers higher than 10^6 cfu/ml. These applications include experiments demanding higher gene transduction efficiency and *in vivo* gene delivery. In addition, crude viral supernatants are not suitable for *in vivo* administration due to various contaminants contained in cell culture supernatant. Thus, the viral supernatant needs to be further concentrated and purified before use.

Traditionally, both recombinant retrovirus and lentivirus have been concentrated via ultracentrifugation. Although the method works well, it requires specific ultracentrifugation equipment and it is technically demanding. In addition, the total viral supernatant volume is limited to the size of ultracentrifugation tubes. Reports have also stated that the ultracentrifugation process has some detrimental physical effects on the recombinant virus.

Due to limitations on the existing technique, there is great necessity for a quick and efficient method to purify and concentrate recombinant retrovirus and lentivirus. Scientists at ABM Inc. have had years of experience with ion exchange-based filter membranes and have successfully developed PuRetro™, the most effective retroviral and lentiviral purification method based on this technology.

Kit Contents

This method proves to be fast, efficient, and reliable. Moreover, it is capable of conveniently processing large volumes of viral supernatant (up to 150ml using the Giga kit). Depending the amount of viral supernatant input and the final elution amount of viral stock, a 50X to 150X fold increase in viral concentration can be achieved using this method.

Table 2. Kit Components

Component	Maxi (2X) (Cat.# G171)	Maxi (5X) (Cat.# G172)	Giga (2X) (Cat.# G173)
Equilibration Buffer	10ml	25ml	20ml
Wash Buffer	20ml	50ml	20ml
Elution Buffer	20ml	50ml	2X10ml
10ml Syringe	2X	5X	2X
20ml Syringe	2X	5X	2X
Syringe Filter	4X	10X	6X
PuRetro™ Purification Unit*	2X	5X	2X

* ***Note:*** *The PuRetro™ has been tested for single use only.*

Additional Materials Required

General Lab Equipment

- Ring stand with clamps
- Ethanol/dry ice bath
- 37°C water bath
- Centrifuge

Fluid Collection

- 150 – 500ml beakers for flow-through waste collection
- Sterile 15ml and 50ml conical tubes

Adenovirus Production

- Tissue culture plates and flasks
- DMEM + 10% fetal bovine serum

Protocol

The following purification protocol is applicable for both recombinant retrovirus and lentivirus that have been pseudotyped with G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) during viral production. For the production of lentivirus and retrovirus, please refer to a manual from any commercial source relating to lentiviral or retroviral expression systems or refer to our manuals in the following web links:

Lentivirus Production:

http://www.abmgood.com/viralexp/Lenti-Easy-His%20Expression%20System.pdf

Retrovirus Production:

http://www.abmgood.com/viralexp/Retro-Easy%20Expression%20System.pdf

A. Lentivirus or Retrovirus Purification

The PuRetro™ filter is designed for single application only. A maximum of 50ml viral supernatant can be processed with Cat.# G171 and G172 (Maxi kits), while 150ml of viral supernatant is the capacity limit for the Giga PuRetro™ Purification Unit (Cat.# G173).

1. **Clear the viral supernatant by centrifugation at 2,000g for 10 minutes.**
2. **Transfer all viral supernatant to a new 50ml conical tube.**
3. **Using a 20ml syringe, complete a pre-filtration step for the supernatant** with syringe filter (included in the kit). For the Giga kit, viral supernatant over 50ml should be split up between two syringe filters. Pre-filtration will decrease viscosity and improve the flow rate of the sample during virus purification steps.

   Note: *The syringe filter is a low protein-binding type to avoid loss of recombinant virus.*
4. **Clamp the syringe column of the PuRetro™ Purification Unit onto a** ring/clamp stand. Place a beaker under the filter unit for waste collection. Alternatively, a syringe pump can be used for the following procedures.

5. **Equilibrate PuRetro™ Purification Unit with 5ml of Equilibration Buffer** or a Giga PuRetro™ Purification Unit with 10ml of Equilibration Buffer. Place the feed tube into a 50ml conical tube containing Equilibration Buffer and pull up the syringe plunger to withdraw 5 or 10ml of Buffer. Dispense the buffer through the purification unit by pressing down lightly on the syringe plunger. The buffer should flow through the filter unit at a rate of 3ml/min (approximately 1 drop per second).

   Note: *Avoid dispensing air through the PuRetro™ Purification Unit. The presence of air in the filter may cause uneven distribution of solutions in the membrane and alter the efficiency of recombinant retrovirus and lentivirus purification. Remove the filter first before depleting the residual air from the syringe.*

6. **Load the viral supernatant onto the PuRetro™ Purification Unit.** Place the feed tube into the conical tube containing the unpurified viral supernatant and pull up the syringe plunger to withdraw. Multiple withdrawals may be required to pass all the viral supernatant through the filter. Dispense the supernatant through the PuRetro™ Purification Unit at a rate of 3ml/min (approximately 1 drop per second).

   Note: *To avoid dispensing air through the filter unit in this and the following step, leave a small volume of supernatant in the syringe. This will not affect the purification process.*

7. **Wash the PuRetro™ unit with 10ml Wash Buffer.** Place the feed tube into the tube containing Wash Buffer and pull up the syringe plunger for withdrawal. Dispense through the PuRetro™ Purification Unit at a rate of 3ml/min (approximately 1 drop per second).

B. Virus Elution

8. **Remove the plunger from a sterile 10ml syringe (provided in the kit).**

9. **Remove the filter from the PuRetro™ Purification Unit.** Attach the filter to the sterile 10ml syringe.

10. **Add 3ml of Elution Buffer to the syringe column.** If the Giga purification unit is used or the elution is to undergo buffer exchange for *in vivo* applications or higher titer concentration, up to 10ml Elution Buffer can be used to increase the elution efficiency.

11. **Replace the plunger in the syringe.**

12. **Elute and discard the first 1.0ml of Elution Buffer into a waste** container. The first 1.0ml is largely comprised of Wash Buffer and should not be kept. Leave the rest of the buffer in the syringe column.
13. **Incubate the filter at room temperature for 5 minutes.** Rest filter on top of a sterile 15ml conical tube to catch any premature elution.
14. **Elute the rest of the Elution Buffer at a rate of 1.0ml/min** (approximately 1 drop every 3 seconds). Use the residual air in the syringe to help expel remaining Elution Buffer from the filter. The eluted buffer contains the purified recombinant lentivirus or retrovirus.

C. Post-Purification Notes

15. **Filter-sterilize viral stock post-purification using a low protein-binding** syringe filter unit (provided in the kit).
16. **The eluted recombinant retrovirus and lentivirus is in a salt buffer but** can be used directly for *in vitro* target cell transduction if the viral dilution is over 5X (i.e. 1.0µl of viral stock to 4µl culture medium). For higher titers of viral stock, viral dilutions less than 5X during transduction, long-term storage of the virus, or proper tonicity for *in vivo* applications, a buffer exchange is required. This can be easily performed using ABM Inc.'s Viral Buffer Exchange Kit (Cat.# G130 on page 15).
17. **1X Storage Buffer: 1X PBS buffer, pH at 7.2.**
18. **Store final viral stock aliquots at -70°C in a non-frost-free freezer.** Avoid repeated freeze-thaw cycles.

D. Determining Recombinant Lentivirus and Retrovirus Titer

For detailed information about recombinant lentivirus and retrovirus titer assay, please refer to our lentivirus and retrovirus expression manuals online.

Lentivirus:
http://www.abmgood.com/viralexp/Lenti-Easy-His%20Expression%20System.pdf
Retrovirus:
http://www.abmgood.com/viralexp/Retro-Easy%20Expression%20System.pdf

E. Transduction of Target Cells

For detailed information about target cell transduction using recombinant lentivirus and retrovirus, please refer to our manuals with the links above.

Viral Stock Buffer Exchange (Cat.# G130)

Cat.# G130 is supplied separately from the recombinant viral purification kits. All recombinant viral purification kits do not contain the viral stock buffer exchange kit.

The eluted recombinant virus is in a salt buffer but can be used directly for *in vitro* target cell transduction if the viral dilution is over 5X (i.e. 1.0µl of viral stock to 4µl culture medium). For higher titers of viral stock or viral dilutions less than 5X during transduction, a buffer exchange is required to make the viral stock suitable for such applications. This can be easily performed using ABM Inc.'s viral buffer exchange kit (Cat.# G130). The exchange filter included in the kit is applicable for all recombinant virus, but the exchange buffer differs.

a. Adenoviral storage buffer: 2.5% glycerol (w/v), 25mM NaCl, and 10mM Tris-HCl, pH 8.0 (Hoganson, et al., 2002).

b. Lentiviral/Retroviral storage buffer: 1X PBS buffer, pH at 7.2.

Protocol

1. **Insert the paddle firmly into the bottom of the sample reservoir.** The "hooks" on the top part of the paddle must rest firmly in the notches on the top of the sample reservoir. For best alignment, turn the reservoir upside–down on the bench-top and gently press the paddle into place.
2. **Attach the filtrate receiver to the bottom of the sample reservoir.**
3. **Top up the eluted viral stock to 15ml using the exchange buffer provided.**
4. **Transfer all the diluted viral samples into the non-membrane side of** the sample reservoir. Attach reservoir cap.
5. **Place the buffer exchange unit into a centrifuge unit that accepts** standard 50ml tubes.

   Note: *Ensure the membrane paddle is parallel with the center of the centrifuge (Figure 2).*
6. **Spin at 3,000g for 45 minutes to achieve desired viral stock volume (2-5ml).**

   Note: *Ensure the centrifuge is counterbalanced.*
7. **Add another 10ml exchange buffer to the non-membrane side of** the sample reservoir and spin again at 3,000g for 35 minutes or more, until the volume within the sample reservoir is approximately 1.0-1.5ml.

   Note: *Ensure the centrifuge is counterbalanced.*

8. **After centrifugation, remove the filtrate receiver from the unit and** screw the concentration cup on.
9. **Check to make sure the "hooks" are in the notches on the sample** reservoir. Return the exchange device to the centrifuge and spin at 3,000g for 3 minutes to collect viral stock.

   Note: *Ensure the centrifuge is counterbalanced.*
10. **Remove the device and unscrew the concentrate cup.**
11. **Attach the 0.2µm syringe filter onto the 3ml syringe (provided in Cat.# G130 kit).**
12. **Transfer viral stock from the concentration cup to the 3ml syringe and** push through the 0.2µm syringe filter for sterilization.
13. **The viral stock is now ready for target cell transduction or aliquot for** long-term storage at –80°C. Alternatively, a small aliquot can be used for titer assay using established protocols or information provided on page 9 for adenovirus and page 14 for retrovirus and lentivirus.

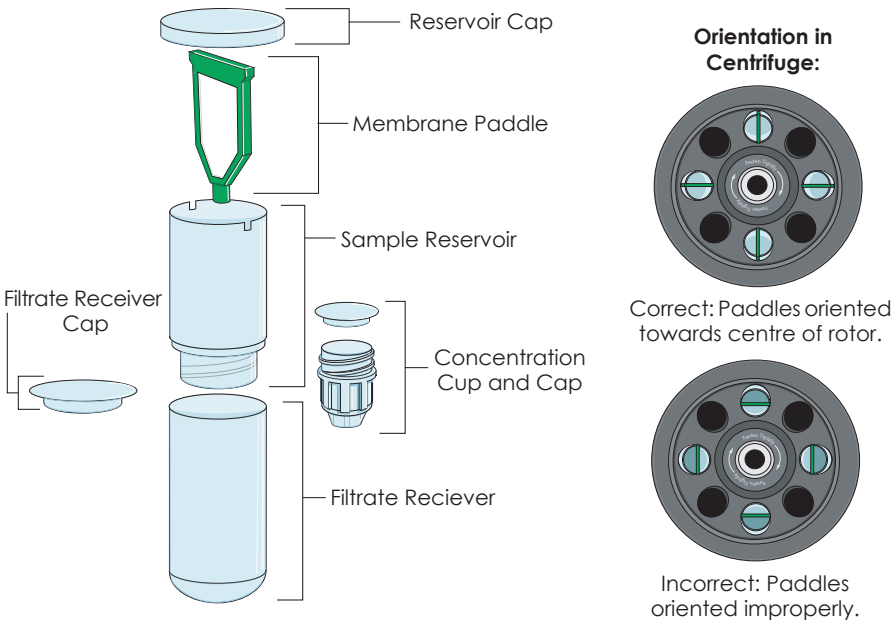

Figure 2: *Buffer Exchange filter components and operation.*

Troubleshooting

Problem	Possible Cause	Solution
Pre-filtration filter clogged	High amount of cellular debris	Avoid disturbing the cellular debris pellet after centrifugation
	Air was pushed into the filter after being wetted	Avoid pushing air into a wet filter
Purification filter is clogged	High amount of cellular debris	Complete the pre-filtration step
Low virus yield	Air entered the filter of the purification unit	Avoid pushing air through the filter
	Flow rates for loading and washing were too fast	Slow the flow rate to 3ml/min (1 drop/second)
	Elution rate was too fast	Slow the flow rate to 1.0ml/min (1 drop/ 3 seconds)
	Remnants of Elution Buffer were left behind in the filter	Expel all of the buffer with the residual air in the syringe
	Procedure was not completed properly	Read and follow the protocols carefully
	The inserted gene is toxic to 293 cells	Use an inducible system or ABM's resistant packaging 293 cells
High amount of cellular DNA	Excess of cellular DNA	Decrease the input amount of viral supernatant

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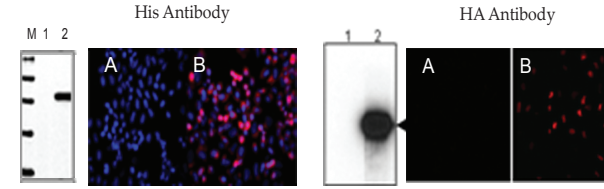

Fig. Lane 1 / A: Untransfected 293 cells. Lane 2 / B: 293 Cells transfected with His or HA tag constructs.

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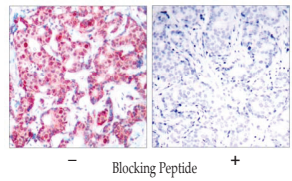
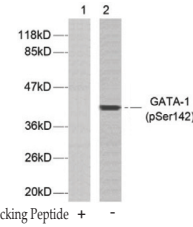


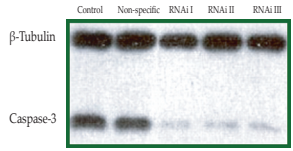
Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using GATA-1 (phospho-Ser142) antibody (# y011041).



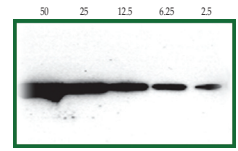
Western blot analysis of extracts from K562 cells using GATA-1 (phospho-Ser142) antibody (# y011041).

Loading Control Antibodies

All loading control antibodies, including GAPDH, Actin, and β-Tubulin antibodies.



Caspase-3 knockdown in 293 cells transfected with 3 different RNAi oligoes.
Loading control was probed with β-tubulin antibody (G098).



293 cell lysates at different amount of total proteins (ug) probed with Anti-β-actin antibody (G046).

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