Ultra-Pure Lentivirus Purification

Ultra-Pure Lentivirus Purification Kit LV998

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Technical Support

Applied Biological Materials, Inc.

Phone: (604) 247-2416
1-866-757-2414

Fax: (604) 247-2414

E-mail: technical@abmGood.com

Biosafety

All lentiviral vectors provided from abm Inc. include the following safety features:

• An enhancer deletion in the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral vector following transduction & integration into the target cell's genomic DNA.

• Utilization of a RSV promoter upstream of 5'ΔLTR allows efficient Tet-independent production of viral RNA.

• The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (Gag/Pol/Rev), and their expression is derived from different plasmids, all lacking packaging signals. The plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus.

• None of the Gag, Pol, or Rev genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.

Despite the safety features discussed above, it is highly recommend- ed that all manipulation with lentiviral vectors, including viral production and transduction, be performed under Biosafety Level 2 (BL-2). All published BL-2 guidelines with proper waste decontamination should be strictly followed. In addition, exercise extra caution when creating lentivirus carrying potentially harmful or toxic genes (e.g. oncogenes). For more information about the BL-2 guidelines and lentivirus handling, refer to "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition. This may be downloaded at: www.cdc.gov/biosafety/publications/bmbl5/index.htm

It is also important to consult with the health and safety officer(s) at your institution for guidelines regarding the use of lentiviruses, and to always follow standard microbiological practices, which include:

• Wear gloves and a lab coat at all times.

• Always work with pseudoviral particles in a Class II culture facility and that all procedures are performed carefully to minimize splashes and aerosols.

• Work surfaces are decontaminated at least once a day and after any spills of viable material.

• All cultures, stocks and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving.

Recombinant lentivirus is widely used in the transduction of a variety of target cells and can be produced by transient transfection, from which viral supernatant can be collected and used for target cell transduction. The titre of viral supernatant by transient transfection is approximately 10^6 IU(infectious units)/ml, which is sufficient for the generation of most stable cell lines *in vitro*. However, there are applications that require higher purity and titres. 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, recombinant lentivirus has 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 to be concentrated is limited to the size of ultracentrifugation tubes. Reports have also stated that the ultracentrifugation process has some detrimental physical effects on the biological activity of purified viral particles.

Due to limitations of the existing technique, there is great need for a quick and efficient method to purify and concentrate recombinant lentivirus. Scientists at abm Inc. have developed the unique Ultra-Pure Lentivirus Purification Kit (LV998), a one of a kind product in the industry. The kit utilizes a proprietary lentivirus binding matrix, binding and elution buffers based on ion-exchange, which minimizes damage to the viral particles while enabling researchers to attain ultra-high titres.

Materials Provided

- LentiBind Buffer A (320ml)
- LentiBind Buffer B (128ml)
- Lenti-Elution buffer (32ml)
- LentiBuffer (40ml)

Other Required Materials

- 13ml sterile centrifuge tube (Sarstedt Cat No. 62.515.006)
- Low protein binding, 0.45µM filter. (Millipore, Cat No. SLHV033RB)
- 100kDa cut-off buffer exchange column (Sartorius Stedim Biotech, Cat No. VS2042)
- Lentifectin (abm Cat No.G074)
- Lenti-Combo mix (abm Cat No. LV003)

Protocol

The 293T cell line is widely used as the optimal cell line for lentivirus production. The health of 293T cells at the time of transfection is a critical factor for the success of lentivirus production. The use of "unhealthy" cells will negatively affect the transfection efficiency, resulting in lower titre lentiviral stocks. For optimal lentivirus production, follow the guidelines below to culture 293T cells before use in transfection:

- Ensure cell viability is greater than 90%.
- Subculture and maintain cells in complete medium containing 0.1mM MEM, 1x Non-Essential Amino Acids, 4mM L-Glutamine, 1mM sodium pyruvate, 500µg/ml Geneticin and 10% FBS.
- Do not allow cells to overgrow before passaging.
- Use cells that have been subcultured for less than 16 passages.
- Make sure 293T cells are free of mycoplasma contamination.

A. Viral Particle Production

1. **One day before transfection (Day 1), plate 293T cells in a 15cm** culture dish so that they will be at 90% density the following day. The number of dishes to be plated is dependent on the final volume and titre of lentivirus to be produced. We recommend making a final viral preparation volume of 1.0ml, but smaller amounts, such as 200µl, can be used to achieve much higher titres. Refer to the following table for the number of 15cm dishes to be plated and the amount of DNA to be used for transfection.

	10^7 IU/ml	10^8 IU/ml	10^9 IU/ml	10^{10} IU/ml
No. of 15cm Dishes	2	10	25	50
Expression Vector	50µg	250µg	625µg	1250µg
Packaging Vector Mix	60µg	300µg	750µg	1500µg
Final Virus Volume	1ml	1ml	1ml	200µl

2. **The following day (Day 2), set up the transfection mix as per the** following table.

Tube A	Tube B
5.0ml Serum Free DMEM	5.0ml Serum Free DMEM
50µg pLentivirus Expression DNA	400µl Lentifectin (Cat. # G074)
120µl Lenti-Combo Mix (Cat. # LV003)	

a. Gently mix the contents in each tube well and incubate for 5 minutes at room temperature.

b. Combine the contents from the tubes A and B, mix thoroughly and incubate for 20 minutes at room temperature to allow the Lentifectin/DNA complexes to form.

c. Add 20ml serum-free DMEM to the complexes followed by gentle mixing.

d. Remove the media from the cells, and add 15ml of Lentifectin/ DNA complexes carefully to each 15cm culture dishes without dislodging cells. Incubate the cells for 5-8 hours at 37°C in a humidified 5% CO_2 incubator. ***Note:*** *293T cells are poorly adhesive to most culture dishes. It is always recommended to add or change medium against the wall of culture dishes to avoid dislodging cells.*

e. 5-8 hrs later, add 1.5ml serum to each plate. Incubate over- night.

3. **The following day (Day 3), aspirate the transfection complexes,** add 20ml fresh complete media, to each 15cm dish.
4. **24 hours later (Day 4), harvest lentiviral supernatant and collect** media into a 250ml centrifuge bottle and proceed to Part B for viral particle binding. Add 20ml fresh complete culture medium to each plate and return the dishes to the incubator. ***Caution: Remember that you are now working with infectious virus at this stage. Follow the recommended guidelines for working with BL-2 organisms.***
5. **On day 5, harvest lentiviral supernatants again from the culture** dishes and collect medium into another 250 ml centrifuge bottle and proceed with virus binding, purifying and concentrating viral supernatant (collected on both day 4 and day 5) using abm's Ultra-Pure Lentivirus Purification Kit (Cat. No. LV998).

B. Virus Purification

1. **On both day 4 and day 5, centrifuge the collected viral supernatant** at 6000g for 10 minutes at 4°C to pellet the cell debris. Transfer supernatant to a new centrifuge container without disturbing the cell debris.
2. **For every 100ml of viral supernatant to be purified, add 20ml of** LentiBind Buffer A and 8.0ml of LentiBind Buffer B. Mix well and shake the mixture on a rotating/orbital shaker (commonly used in Western Blot applications) at 200-300rpm at 4°C overnight (for day 4 collection).
3. **For day 5 viral supernatant collection, allow the binding for 3** hours only and then combine both collections before proceeding to step 4.
4. **Centrifuge at 7000g at 4°C for 30 mins. to collect the viral particles.**
5. **Discard supernatant completely without disturbing the pellet,** and spin briefly again if necessary to remove any residual liquid.
6. **Add 8.0ml Lenti-Elution buffer to resuspend the viral pellet completely** with a transfer pipette and transfer the viral suspension to a 10ml sterile tube that is resistant to 10,000g during centrifugation in the following step (Sarstedt Cat No. 62.515.006). Shake the tubes at 4°C for 30min to elute viral particles from LentiBind Buffer A.

7. **After incubation, spin at 10,000rpm for 20 min to separate viral** particles from the binding matrix.
8. **Transfer the supernatant (viral particles) to a 10ml syringe and** filter preparation through a 0.45μM filter (Millipore, Cat No. SLHV033RB) to further clarify the preparation.
9. **Perform buffer exchange for the viral preparation to get rid of** the high salt concentrations carried over from LentiBind Buffer B using a 100kDa cut-off protein concentrating column (Sartorius stedim biotech, Cat No. VS2042).
10. **Spin the column at 3000rpm at 20°C until the viral supernatant** volume is close to 1-2ml. To speed up the process, use 1ml transfer pipette to re-suspend the viral preparation every 30 minutes.
11. **Add 10ml LentiBuffer and spin the column again at 3000rpm at** 20°C to exchange for final buffer until the final volume is 200μl-1.0ml depending on viral titre desired. To speed up the process, use 1ml transfer pipette to re-suspend the supernatant every 30 minutes.
12. **Transfer the final viral preparation from the buffer exchange col-**umn to a 1-3ml syringe and sterilize it through a 0.45μM filter. Make sure to pre-wet the filter with 100μl of LentiBuffer to prevent virus loss caused by residual liquid left in the filter.
13. **Final lentivirus preparations can either be used immediately for** cell transduction or aliquoted into smaller volumes (10-20μl) for long-term storage at -75°C. The Ultra-Pure Lentivirus Purification Kit (LV998) can be stored at room temperature.

Contact Information

Applied Biological Materials Inc.

Website:
www.abmGood.com

Phone:
(8:30am-4:30pm PST M-F)
Toll Free: 1-866-757-2414
Local: (604) 247-2416
Fax: (604) 247-2414 (24Hr.)

Address:
Suite #8-13520 Crestwood Place
Richmond, BC
Canada V6V 2G2

Email:
General Information:
info@abmGood.com

Order Products:
order@abmGood.com

Technical Support:
technical@abmGood.com

siRNA:
siRNA@abmGood.com

Business Development:
bd@abmGood.com

Distributors

North America

Canada
Applied Biological Materials Inc.
Tel: (604) 247-2416 / 1-866-757-2414
Fax: (604) 247-2414
www.abmGood.com

United States
Applied Biological Materials Inc.
Tel: (604) 247-2416 / 1-866-757-2414
Fax: (604) 247-2414
www.abmGood.com

Mexico
Quimica Lavoisier S.A. de C.V.
Tel: 52-333-848-8484
Email: informes@lavoisier.com.mx
www.lavoisier.com.mx

International

Australia
Biosensis Pty Ltd.
Tel: +61 43 166 5519
Email: sales@biosensis.com
www.biosensis.com

Belgium
Gentaur
Tel: 32 2 732 5688
Email: ea@gentaur.com
www.gentaur.com

France
Gentaur
Tel: 01 43 25 01 50
Email: ea@gentaur.com
www.gentaur.com

Germany
BioCat GmbH
Tel: +49 (0) 6221-714-1516
Email: info@biocat.com
www.biocat.com

India
G Biosciences
Tel: 0120-432-3330
Email: rohit@gbiosciences.com
www.GBiosciences.com

Israel
BioConsult
Tel: 972-(0)2-566-7043
Email: sales@bioconsult.co.il
www.bioconsult.co.il

Italy
MICROTECH s.r.l.
Tel: +39-0816107435
Email: microtech@microtech.eu
www.microtech.eu

Japan
Cosmo Bio Co. Ltd.
Tel: 03-5632-9610/9620
Email: mail@cosmobio.co.jp
www.cosmobio.co.jp

Singapore
Bio-REV PTE
Tel: (65) 6273-3022
Email: allan@bio-rev.com
bio-rev.com

South Korea
CMI Biotech
Tel: 02 444 7101
Email: cmibio@cmibio.com
www.cmibio.com

Taiwan
Interlab Co. Ltd.
Tel: +886-2-2736-7100
Email: service@interlab.com.tw
www.interlab.com.tw

United Kingdom
NBS Biologicals Ltd.
Tel: +44 (0)1480 433875
Email: info@nbsbio.co.uk
www.nbsbio.co.uk