



Retro-Concentin™ Virus Precipitation Solution (5×)

Cat. # RV100A-1

User Manual

Store kit at 4°C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 1-20100428)

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List of Components

Each Retro-Concentin™ bottle provides enough material to precipitate virus from 400 ml of viral supernatant.

100 ml Retro-Concentin™ Virus Precipitation Solution

The kits are shipped at room temperature or on blue ice and should be stored at +4°C upon receipt. Properly stored kits are stable for 1 year from the date received.

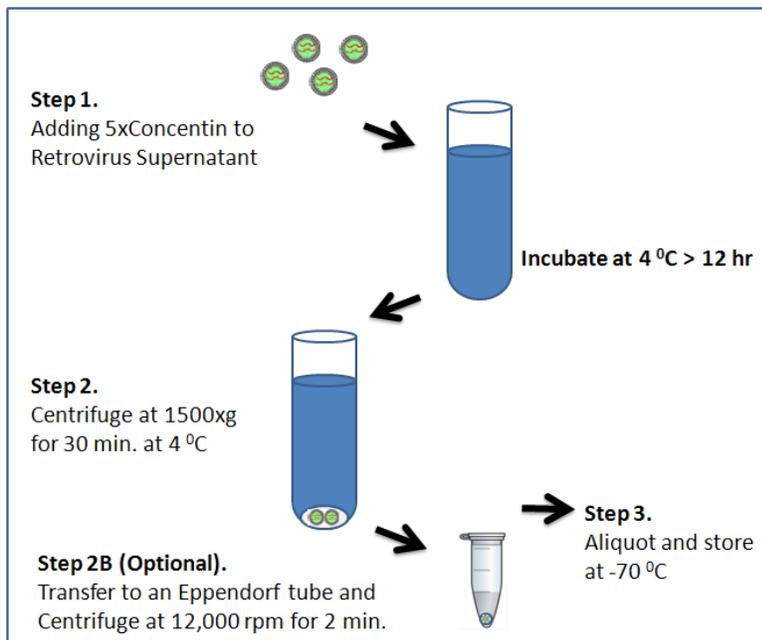
I. Introduction

Retroviral gene transfer is a technique for efficiently introducing stable, heritable genetic materials into the genome of any dividing host cell types. Moloney murine leukemia viral (MMLV)-based gene delivery technology is the most widely used retroviral vector in gene delivery, gene therapy due to its ability to stably integrate its transgene into host chromosomal DNA with low immunogenicity.

Generally, retrovirus is fragile and subject to inactivation to harsh environment and prolonged unfriendly procedures such as affinity column purification and dialysis. Therefore, ultracentrifugation or other multistep protocols aimed to concentrate and purify viruses may cause damage to retroviral particles.

Retro-Concentin™ does not involve ultracentrifugation or complicated procedures. Instead, retroviruses are directly pelleted from culture medium with simple one/two steps of low speed centrifugations. In addition, some of ingredients in the concentration solution may also stabilize the viruses, which would provide a protection against the viral inactivation. Each preparation can handle up to 200 ml of retroviral supernatant (most centrifuges) and resulting pellet can be dissolved in a desired volume to meet your experiment requirement.

Flowchart



II. Protocol

A. Protocol

Retroviral Particle Concentration by Retro-concentin™ Precipitation

Supernatant of cultured 293TN (SBI, Cat. # LV900A-1) or other producer cell lines such as PLAT-E (engineered retroviral packaging cells) is collected for viral precipitation. After collection of retrovirus-containing supernatant, the following protocol allows for precipitation and concentration of retrovector particles, with a 1 to 3 log increase in final titer.

1. Collect supernatant and centrifuge at $3000 \times g$ for 15 minutes to remove cells and cell debris. Supernatant may be filtered through a $0.45 \mu\text{m}$ PVDF filter to further eliminate cellular debris. Please note that filtration may decrease the amount of virus in the supernatant, and should be reserved for clarifying supernatants that will be used to transduce target cells that are sensitive to cell debris.
2. Transfer the above supernatant to a sterile vessel and add 1 volume of Retro-Concentin Virus Precipitation Solution to every 4 volumes of retrovirus-containing supernatant. The retroviral Precipitation Solution is a 5x solution.
3. Refrigerate overnight (at 4°C . for at least 12 hours). Note: Retroviral particle-containing supernatants mixed with Retro-Concentin Virus Precipitation Solution are stable for up to 2 weeks at 4°C .
4. Centrifuge supernatant/Retro-Concentin mixture at $1500 \times g$ for 30 minutes. After centrifugation, the retroviral particles may appear as a beige or white pellet at the bottom of the vessel.
5. Aspirate supernatant. Spin down residual Retro-Concentin solution by centrifugation at $1500 \times g$ for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated retroviral particles in pellet.
6. Re-suspend retroviral pellet in 1/10 to 1/500 of original volume using sterile Phosphate Buffered Saline (PBS) or DMEM containing 25mM HEPES buffer.
7. Aliquot in cryogenic vials and store at -70°C until ready for use.

Precipitation of retroviral particles from large volumes can be achieved by using the Corning 250 mL polypropylene centrifuge tube (Cat. # 430776), following manufacturers instructions.

B. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

<http://www.systembio.com>

For additional information or technical assistance, please call or email us at:

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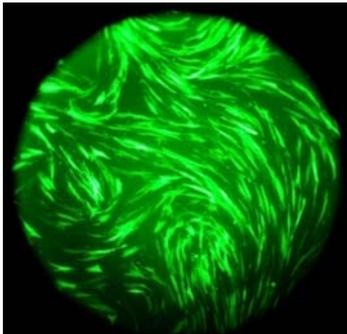
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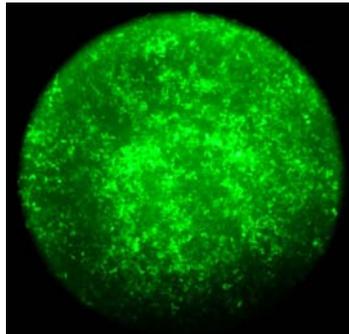
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III. Example of Results

The following figures demonstrate typical transduction experiments using retroviral particle prepared using Treto-Concentin™. Cells transduced show normal morphology and no obvious cytotoxicity is observed. (The data should not be used to interpret actual results. One should use the data below for reference only.) High titer virus particles prepared using Retrocentin were successfully used for generating induced pluripotent stem cells at SBI.



Human Foreskin Fibroblasts
(Primary Culture)



Human Embryonic Kidney Cells
(Cell line)

Cells were transduced using a GFP expression retroviral construct

IV. Licensing and Warranty Statement

Limited Use License

Use of the Retro-Concentin Virus Precipitation Solution (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a credit. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

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