ExCell Bio
依科赛生物

ExCell Bio

Retro-Concentin Virus Precipitation Solution（5X）

User Manual

Catalog Number　EMB100A-1　　100mL

Introduction

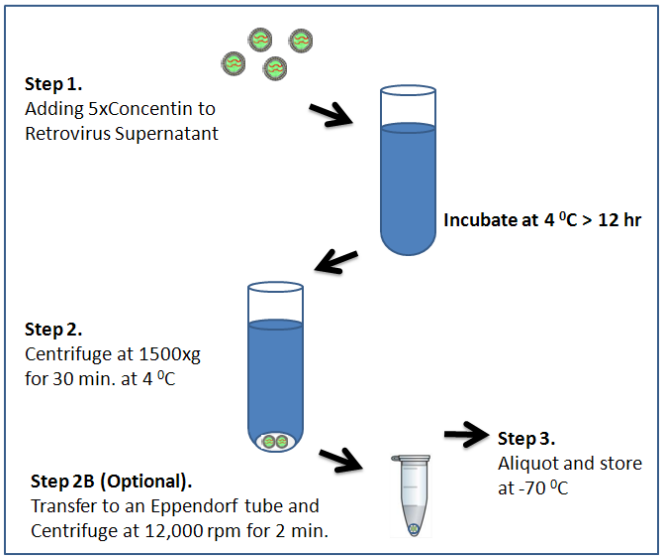
Retroviral gene transfer is a technique for efficiently introducing stable, heritable genetic materials into the genome of any dividing host cell types. Moloney murine leukemia viral (MMLV)-based gene delivery technology is the most widely used retroviral vector in gene delivery, gene therapy due to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

Generally, Retro-Concentin Virus 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 Virus does not involve ultracentrifugation or complicated procedures. Instead, Retro-Concentin Viruses are directly pelleted from culture medium with simple one/two steps of low speed centrifugations. In addition, the concentration solution may also stabilize the viruses, which may provide an advantage over other methods. Each preparation can handle up to 200 ml of retroviral supernatant (most centrifuges) and resulting pellet can be in a desired volume to meet your experiment requirement.

Flowchart

Flowchart



Shipping & Storage

Shipping： Room temperature

Storage ： 4°C

Shelf Life： 12 months from date of receipt with proper storage

Protocol

Retroviral Particle Concentration by Retro-Concentin Virus Precipitation

Supernatant of cultured 293TN or other 293 producer cell lines such as PLAT-E (engineered retroviral package cells) is collected for viral precipitation. After collection of Retro-Concentin Virus-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 × g for 15 minutes to remove cells and cell debris. Supernatant may be filtered through a 0.45 μm 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 supernatant to a sterile vessel and add 1 volume of Retro-Concentin Virus Precipitation Solution to every 4 volumes of Retro-Concentin Virus-containing supernatant. The retroviral Precipitation Solution is a 5× solution.

3. Refrigerate overnight (at 4 ℃. at least 12 hours). 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 Virus mixture at 1500 × 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 Virus solution by centrifugation at 1500 × g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated retroviral particles in pellet.

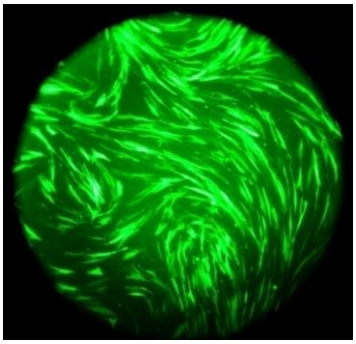
6. Re-suspend retroviral pellet in 1/10 to 1/500 of original volume using sterile Phosphate Buffered Saline (PBS) or DMEM containing 25mM HEPES buffer.

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, following manufacturers instructions.

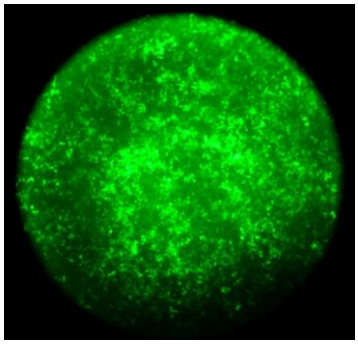
Data Analysis

The following figures demonstrate typical transduction experiments using retroviral particle prepared using Retro-Concentin Virus. 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.



Human Foreskin Fibroblasts

(Primary Culture)



Human Embryonic Kidney Cells

(Cell line)

Cells were transduced using a GFP expression retroviral construct