Product Manual

ViraDuctin™ Retrovirus Transduction Kit

Catalog Number

RV-200	40 transductions (24-well plate)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Retroviral gene transfer is a technique for efficiently introducing stable, heritable genetic material into the genome of any dividing cell type. Replication-incompetent retrovirus is usually produced through transfection of the retroviral vector into a packaging cell line. Retroviruses are classified according to the receptors used to enter host cells. Ecotropic virus can recognize a receptor found on only mouse and rat cells. Amphotropic virus recognizes a receptor found on a broad range of mammalian cell types.

Retrovirus usually has lower titer than lentivirus which leads to poor gene transfer. The rate at which retroviral vectors bind to and infect cells is mostly controlled by diffusion. During retrovirus infection, only a small fraction of the retroviral vectors can transduce target cells. Virion adsorption is the limiting step of this process. Another cause of poor gene transfer of retrovirus is the presence of transduction inhibitors in retroviral supernatants such as proteoglycans and glycosaminoglycans. The use of polycations, such as Polybrene®, is standard in many retroviral infection protocols owing to the observations of improved infection efficiency by neutralizing the electrostatic repulsion between virion and cell membranes, but the transduction inhibitors still remain in the retroviral supernatant during infection.

ViraDuctin™ Retrovirus Transduction Kit is a proprietary formulation for the rapid purification of retrovirus from inhibitors of transductions. The ViraDuctin™-retrovirus complexes will also quickly sediment onto target cells resulting in fast delivery of the viruses than simple diffusion. Both removal of transduction inhibitors and quick delivery of viruses to cells result in higher gene transfer.

ViraDuctin™ Retrovirus Transduction Kit provides the following advantages:

- Higher transduction efficiency in many cell types compared to reagents such as Polybrene®.
- Rapid purification and removal of transduction inhibitors.
- Quick delivery of viruses to cells.
- Ideal for transduction of nonpermissive cells such as primary cells and stem cells.

Related Products

1. AAV-200: ViraDuctin™ AAV Transduction Kit
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
3. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
4. RV-101: Platinum-E Retroviral Packaging Cell Line, Ecotropic
5. RV-201: ViraDuctin™ Retrovirus Transduction Kit, 200 Transductions
6. VPK-120: QuickTiter™ Retrovirus Quantitation Kit
7. VPK-300: Platinum Retroviral Expression System, Ecotropic
8. VPK-301: Platinum Retroviral Expression System, Amphotropic
9. VPK-302: Platinum Retroviral Expression System, Pantropic

Kit Components

1. ViraDuctin™ Retrovirus Transduction Reagent A (100X) (Part No. 320004): One sterile tube – 200 µL
2. ViraDuctin™ Retrovirus Transduction Reagent B (100X) (Part No. 320005): One sterile tube – 200 µL
3. ViraDuctin™ Retrovirus Transduction Reagent C (8X) (Part No. 320006): Two sterile tubes – 1.5 mL each

Materials Not Supplied

1. Retroviral Stock Solution
2. Cells and cell culture growth medium
3. 37ºC Incubator

Storage

Store all kit components at 4ºC. DO NOT FREEZE.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Transduction Protocol

The following transduction protocol is written for a 24-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats.

Culture Dish	96-well	24-well	12-well	6-well	60-mm	10-cm
Retrovirus/Culture Media (µL)	100	500	1000	2000	5000	10000
Reagent A (100X) (µL)	1	5	10	20	50	100
Reagent B (100X) (µL)	1	5	10	20	50	100
Final Transduction Volume (µL)	102	510	1020	2040	5100	10200
Reagent C (1X) (µL)	100	500	1000	2000	5000	10000

Table 1: Dispensing Volumes of Different Plate Formats

I. Transduction of Adherent Cells

1. The day before transduction, trypsinize and count the cells, plating 0.2-2 x 10^5 cells in 0.5 mL complete culture medium per well of a 24-well plate. Incubate cells at 37°C overnight.

2. On the day of transduction, transfer 0.5 mL of your retroviral supernatant in a sterile tube. Add 5 µL of ViraDuctin™ Retrovirus Transduction Reagent A (100X) and mix by inverting. Immediately add 5 µL of ViraDuctin™ Retrovirus Transduction Reagent B (100X) and mix by inverting.
3. Incubate 30 minutes at 37°C.
4. Centrifuge for 10 minutes at 12,000 g. Carefully remove and discard supernatant, resuspend the pellet in 0.5 mL of fresh culture medium used for your target cells.
5. Remove culture medium from the 24-well plate and apply all 0.5 mL of the retrovirus-ViraDuctin™ mixture to cells.
6. Incubate at 37°C overnight.
7. Dilute the appropriate amount of ViraDuctin™ Retrovirus Transduction Reagent C (8X) to 1X with complete culture medium (for example, add 70 µL of 8X Reagent C to 490 µL of complete culture medium).
8. Remove the media containing Retrovirus-ViraDuctin™ complexes and replace with 0.5 ml of complete culture medium.
9. To completely remove the transduction complex, remove the culture medium and replace with 500 µL of the diluted ViraDuctin™ Retrovirus Transduction Reagent C (1X) in each well; gently rock the plate for 30-60 seconds. IMMEDIATELY aspirate the medium containing ViraDuctin™ Retrovirus Transduction Reagent C and replace with 0.5 ml of complete culture medium. Wash twice with complete culture medium to remove any residue complex.
10. 48-72 hrs after transduction, proceed with desired method of detection. To select stable cell clones, replace medium with fresh medium containing antibiotic every 3-4 days until antibiotic-resistant colonies can be identified.

II. Transduction of Suspension Cells

1. The day before transduction count the cells, plating 0.2-2 x 10^5 cells in 0.5 mL complete culture medium per well of a 24-well plate. Incubate cells at 37°C overnight.
2. On the day of transduction, transfer 0.5 mL of your retroviral supernatant in a sterile tube. Add 5 µL of ViraDuctin™ Retrovirus Transduction Reagent A (100X) and mix by inverting. Immediately add 5 µL of ViraDuctin™ Retrovirus Transduction Reagent B (100X) and mix by inverting.
3. Incubate 30 minutes at 37°C.
4. Centrifuge for 10 minutes at 12,000 g. Carefully remove and discard supernatant, resuspend the pellet in 0.5 mL of fresh culture medium used for your target cells.
5. Pellet your suspension cells for 5 minutes at 1000 g. Remove supernatant and resuspend pellet in 0.5 mL of the medium containing retrovirus from step 4.
6. Incubate at 37°C overnight.
7. Dilute the appropriate amount of ViraDuctin™ Retrovirus Transduction Reagent C (8X) to 1X with complete culture medium (for example, add 70 µL of 8X Reagent C to 490 µL of complete culture medium).
8. Centrifuge cells for 5 minutes at 1000 g and remove supernatant. Resuspend pellet in 0.5 mL of Reagent C/medium mixture from step 7.
9. IMMEDIATELY centrifuge for 5 minutes at 1000 g. Aspirate the supernatant containing Reagent C and replace with 0.5 ml of complete culture medium. Repeat twice to remove any residue complex.

10. 48-72 hrs after transduction, proceed with desired method of detection. To select stable cell clones, replace medium with fresh medium containing antibiotic every 3-4 days until antibiotic-resistant colonies can be identified.

Recent Product Citations

1. Gandhi, M. et al. (2012). Homologous chromosomes make contact at the sites of double-strand breaks in genes in somatic G0/G1-phase human cells. *PNAS*. **109**:9454-9459.
2. Miyoshi, N. et al. (2010). Defined factors induce reprogramming of gastrointestinal cancer cells. *PNAS* **107**:40-45.

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