Cat. # T110A

For Research Use

TaKaRa

RetroNectin® Dish

(RetroNectin Pre-coated Dish, 35 mmφ)

Product Manual

v201705

Table of Contents

I. Description

RetroNectin Dish is a culture dish (35 mmφ) that has been coated with RetroNectin reagent (Cat. #T100A/B)*1. RetroNectin reagent is a recombinant human fibronectin fragment (CH-296) that is composed of three functional domains: the cell-binding domain (C-domain), heparin-binding domain (H-domain), and CS-1 sequence. The fragment enhances viral-mediated gene transduction by aiding the co-localization of target cells and virions. Specifically, virus particles bind RetroNectin reagent via interaction with the H-domain, and target cells bind mainly through the interaction of cell surface integrin receptor VLA-5 and VLA-4 with the fibronectin C-domain and CS-1 site, respectively. Through facilitating close proximity, RetroNectin reagent can enhance retroviral-mediated gene transfer to target cells expressing integrin receptors VLA-4 and/or VLA-5.*2

There are two RetroNectin-mediated infection protocols: the supernatant (SN) infection method and the RetroNectin-bound virus (RBV) infection method.[5),*3] With the SN infection method, cells are mixed with virus supernatant and loaded on a RetroNectin-coated plate. In the RBV method, the retrovirus is first bound to the RetroNectin-coated plate, and cells are added after removing the retrovirus supernatant. Removal of the supernatant reduces inhibitory molecules (e.g., molecules secreted from the virus producing cells such as proteoglycans and/or viral envelope proteins) that can reduce the efficiency of viral-mediated gene transduction.

*1 Not available in all geographic locations. Check for availability in your region.

*2 RetroNectin reagent can also enhance lentiviral-mediated gene transfer.

*3 Both methods can be used for efficient gene transduction. Although the RBV infection method is widely applicable, some modification might be required depending on the target cells, vectors, and/or target genes.

II. Components

RetroNectin Dish (RetroNectin Pre-coated Dish, 35 mmφ)	10 dishes

Note: The dish may contain crystalized salt; this will not effect use.

III. Storage 4℃

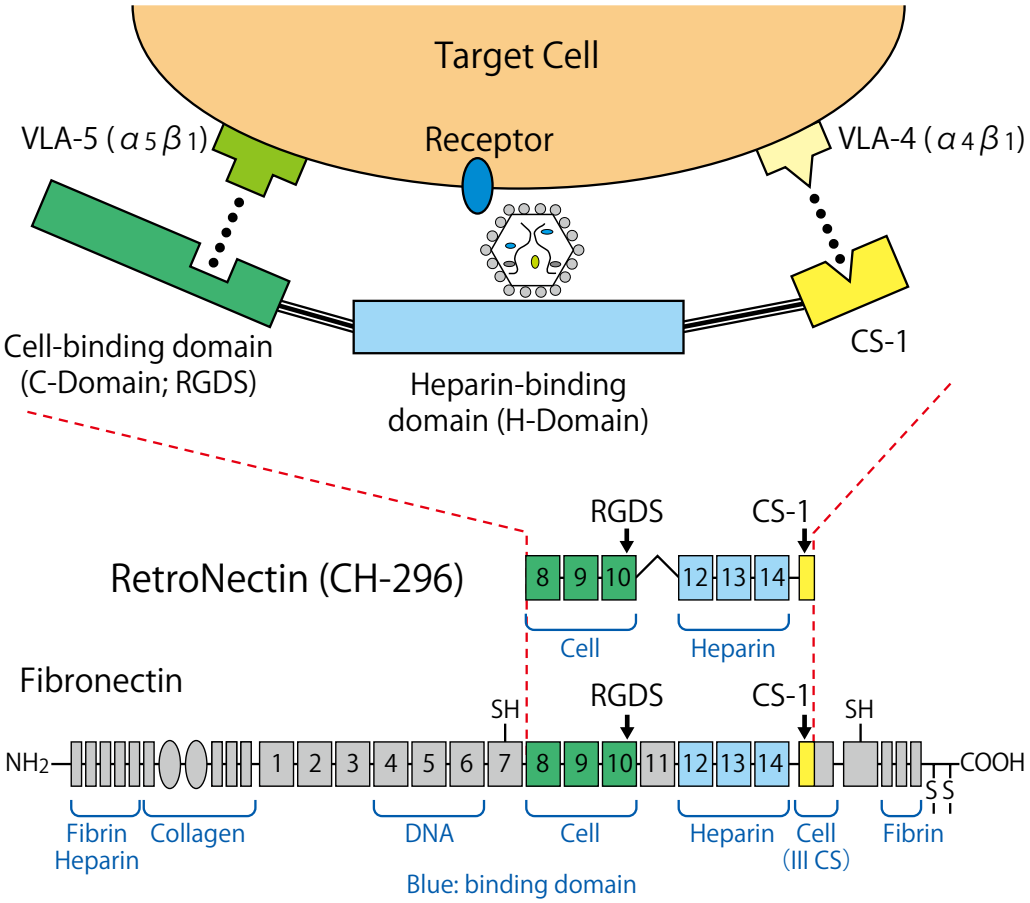

Figure 1. The hypothesized mechanism of RetroNectin-mediated transduction.

The cell binds to the CS-1 site via VLA-4, and to the C-domain via VLA-5. The viral particle can bind to the H-domain of RetroNectin. These interactions increase the localized concentrations of cells and viral particles, an effect that is thought to enhance gene transduction.

IV. Materials Required but not Provided

[Equipment]

1. Electric pipetter
2. Pipetter
3. Sterile pipettes
4. Sterile filter tips
5. Safety cabinet or clean work station
6. Microscope
7. CO_2 incubator

[Reagents]

1. Sterile PBS (-)
2. HBSS/Hepes (Hank's Balanced Salt Solution supplemented with 2.5% (v/v) 1 M Hepes)
3. 2% bovine serum albumin (BSA Fraction V)/PBS

V. Gene Transduction

There are two methods of gene transduction using RetroNectin reagent: RetroNectin-bound virus (RBV) infection method (Section A) and supernatant (SN) infection method (Section B). In the RBV infection method, retroviral particles are first bound to the plate coated with RetroNectin, and the target cells are added after removing the virus supernatant. In the SN infection method, the virus solution and target cells are mixed and then added to the RetroNectin Dish.

When virus solution is directly used for virus infection without purification, the gene transduction efficiency may be reduced because of contaminating molecules that inhibit infection. In such cases, the RBV infection method is recommended. With this method, inhibitory molecules can be removed by binding viruses to RetroNectin reagent and removing the supernatant.

Note: Using a transient production system, such as the Retrovirus Packaging Kit Eco (Cat. #6160)*/Retrovirus Packaging Kit Ampho (Cat. #6161)* and the pDON-AI-2 Neo DNA (Cat. #3653)/pDON-AI-2 DNA (Cat. #3654), retroviral vectors can be prepared in one week.

* Not available in all geographic locations. Check for availability in your region.

VI. Transduction Protocol

A. RetroNectin-Bound Virus (RBV) Infection Method

A-1. Preparation of Virus-Bound Plate

1. Add retrovirus supernatant at 125 - 250 μl/cm^2 to a RetroNectin Dish.
2. Incubate for 4 to 6 hours at 32℃ or 37℃ in a 5% CO_2 incubator to promote binding of the virus particles with RetroNectin reagent. During this incubation, retrovirus vector will bind to the H-domain of RetroNectin reagent.
3. Discard the supernatant, but do not allow the plate to dry. Wash the plate with an appropriate volume of PBS or PBS containing 0.1 - 2% albumin (BSA or HSA) to remove undesirable substances in the retrovirus supernatant.

A-2. Virus Infection

Prepare the target cells while the retrovirus particles are binding to the RetroNectin Dish. It is important that the target cells be in logarithmic growth phase and express integrin receptors VLA-4 and/or VLA-5. When using hematopoietic stem cells, pre-stimulation with cytokine may be necessary. The cytokine type should be determined based on your specific research protocols. Examples are cited in references 3 and 5.

1. Collect the target cells and count the number of living cells. Then suspend the cells in the growth medium at a concentration of 0.2 - 1 x 10^5 cells/ml.
2. Remove the solution from the virus bound plates prepared in Step A - 1. After removal of the solution, do not allow the plate to dry. Immediately add target cells in growth medium at the concentration of 0.5 - 2.5 x 10^4 cells/cm^2. Although the optimal cell density depends on cell size and growth rate, the initial cell density should allow the cells to be actively growing or nearly confluent when analyzed 2 - 3 days after transduction. When infecting more cells, you may increase the cell density, but the cells will need to be subcultured after gene transduction.
3. Incubate at 37℃ with 5% CO_2 for 2 - 3 days.
4. Collect both non-adherent and adherent cells:
   (1) Transfer the supernatant to a centrifuge tube.
   (2) Recover remaining non-adherent cells by washing the plate with PBS.
   (3) Dissociate adherent cells from the plate with Cell Dissociation Buffer (Thermo Fisher Scientific), an enzyme free solution, or trypsin-EDTA following the manufacturer's instructions.

   Note: For many cell types, adherent cells may be collected by pipetting only.
   (4) Combine the cells obtained from Steps (1) - (3) in the same tube, and centrifuge to recover the cells.
   (5) Wash the cells with HBSS/Hepes twice, collecting the cells by centrifugation. Suspend the cells in HBSS/Hepes* for further analysis.
   * Any buffer or medium suitable for downstream application of the cells can be also used for resuspension.

B. Supernatant (SN) Infection Method

When the virus stock solution is used, the RBV method described in A is recommended, but if a 4-fold dilution or more is used, either the RBV or SN method may be used as equivalent gene transduction efficiency will be obtained. The time required for virus infection is much shorter with the SN method than with the RBV method.

1. Suspend the target cells in virus solution that has been diluted with growth medium to prepare the cell suspension.
2. Add the cell suspension to the RetroNectin Dish at the concentration of 0.5 - 2.5 x 10^4 cells/cm^2. Although the optimal cell density depends on cell size and growth rate, the initial cell density should allow the cells to be actively growing or nearly confluent when gene expression is analyzed 2 - 3 days after transduction. When infecting more cells, you may increase the cell density, but the cells will need to be subcultured after gene transduction.
3. Incubate at 37℃ with 5% CO_2 for 2 - 3 days.
4. Collect both non-adherent and adherent cells:
   (1) Transfer the supernatant to a centrifuge tube.
   (2) Recover remaining non-adherent cells by washing the plate with PBS.
   (3) Dissociate adherent cells from the plate with Cell Dissociation Buffer (Thermo Fisher Scientific), an enzyme free solution, or trypsin-EDTA following the manufacturer's instructions.

   Note: For many cell types, adherent cells may be collected by pipetting only.
   (4) Combine the cells obtained from Steps (1) - (3) in the same tube, and centrifuge to recover the cells.
   (5) Wash the cells with HBSS/Hepes twice, collecting the cells by centrifugation. Suspend the cells in HBSS/Hepes* for further analysis.
   * Any buffer or medium suitable for downstream application of the cells can be also used for resuspension.

VII. References

1) Kimizuka F, Taguchi Y, Ohdate Y, Kawase Y, Shimojo T, Hashino K, Kato I, Sekiguchi K, and Titani K. Production and characterization of functional domains of human fibronectin expressed in *Escherichia coli*. *J Biochem*. (1991) **110**: 284-291.
2) Hanenberg H, Xiao XL, Dilloo D, Hashino K, Kato I, and Williams DA. Colocalization of retrovirus and target cells on specific fibronectin fragments increases genetic transduction of mammalian cells. *Nat Med*. (1996) **2**: 876-882.
3) Hanenberg H, Hashino K, Konishi H, Hock RA, Kato I, and Williams DA. Optimization of fibronectin-assisted retroviral gene transfer into human $CD34^+$ hematopoietic cells. *Hum Gene Ther*. (1997) **8**: 2193-2206.
4) Pollok KE, Hanenberg H, Noblitt TW, Schroeder WL, Kato I, Emanuel D, and Williams DA. High-efficiency gene transfer into normal and adenosine deaminase-deficient T lymphocytes is mediated by transduction on recombinant fibronectin fragments. *J Virol.* (1998) **72**: 4882-4892.
5) Chono H, Yoshioka H, Ueno M, and Kato I. Removal of inhibitory substance with recombinant fibronectin-CH-296 plates enhances the retroviral transduction efficiency of $CD34^+$ $CD38^-$ bone marrow cells. *J Biochem*. (2001) **130**: 331-334.

VIII. Related Products

[Retroviral vectors]
pDON-5 DNA (Cat. #3658)
pDON-5 Neo DNA (Cat. #3657)
pDON-AI-2 Neo DNA (Cat. #3653)
pDON-AI-2 DNA (Cat. #3654)
pMEI-5 Neo DNA (Cat. #3655)
pMEI-5 DNA (Cat. #3656)

[Preparation of Recombinant Retroviral Particles]
Retrovirus Packaging Kit Ampho (Cat. #6161)*
Retrovirus Packaging Kit Eco (Cat. #6160)*
Retro-X™ System (Cat. #631508)
Retro-X™ Universal Packaging System (Cat. #631530)

[Lentiviral Vectors and Vector Systems]
Lenti-X™ Expression System (Cat. #632164)

[Preparation of Recombinant Lentiviral Particles]
Lenti-X™ 293T Cell Line (Cat. #632180)
Lenti-X™ Packaging Single Shots (VSV-G) (Cat. # 631275)
Lenti-X™ HTX Ecotropic Packaging System (Cat. #631251)

[Other]
RetroNectin® (Recombinant Human Fibronectin Fragment) (Cat. #T100A/B)*
RetroNectin® GMP grade Recombinant Human Fibronectin Fragment CH-296 (Cat. #T201)*
Retrovirus Titer Set (for Real Time PCR) (Cat. #6166)
Retro-X™ qRT-PCR Titration Kit (Cat. #631453)
Lenti-X™ qRT-PCR Titration Kit (Cat. #631235)*

* Not available in all geographic locations. Check for availability in your region.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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