OZYME

TaKaRa

Improve Viral Transductions with RetroNectin® Reagent

Recombinant fibronectin molecule from Takara Bio colocalizes virus and cells to facilitate gene transfer

- **Improves gene transfer efficiency in hard-to-infect cell types and stem cells**
- **Multivalent molecule simultaneously binds virus particles and cell surface proteins, maximizing cell-virus contact**
- **Ideal for use with Lenti-X™ and Retro-X™ Expression Systems**
- **For more information visit www.clontech.com or www.takarabio.com**

Retroviral and lentiviral vectors offer stable and effective gene delivery to many different types of cells. However, some cell types such as stem cells and cells cultured in suspension are often poorly transduced using standard methods. Takara's **RetroNectin® Reagent** for retrovirus-mediated gene transfer can dramatically increase retroviral and lentiviral transduction efficiency; especially in cells grown in suspension culture (e.g. lymphocytes) and in hematopoietic stem cells (1, 2). RetroNectin Reagent is available in vials of 0.5 mg each (1- or 5-vial sizes) or in ready-to-use **RetroNectin® Precoated Dishes** (35 mm).

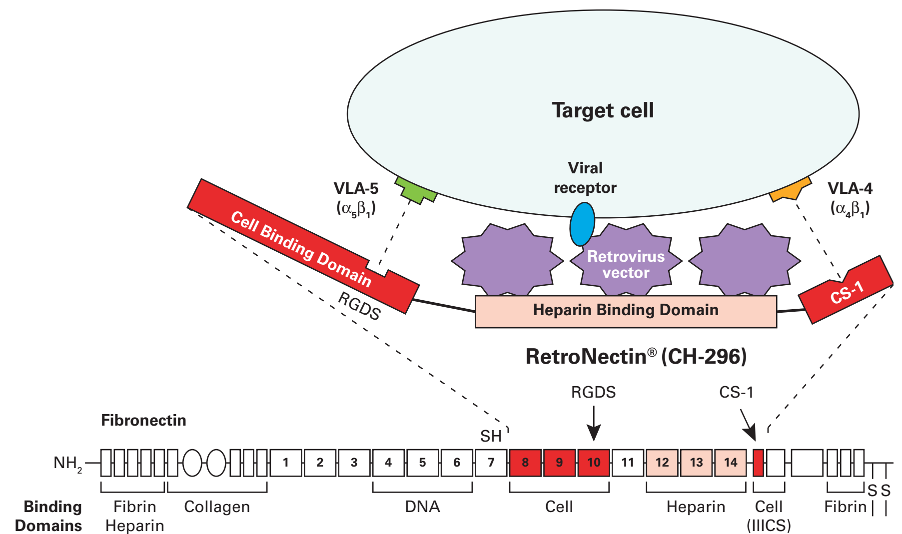

Figure 1. RetroNectin greatly enhances virus-mediated gene transduction. RetroNectin is a chimeric recombinant peptide consisting of 3 functional domains derived from human fibronectin, that bind either cell surface proteins or virus particles. The enhancement is likely due to the colocalization of virus and cells on RetroNectin molecules. RetroNectin is most effective when viruses are first allowed to bind to RetroNectin-coated plates (or other cell growth substrates), followed by the addition of target cells.

What is RetroNectin?

Fibronectins (FN) comprise a family of multifunctional adhesive glycoproteins found in the extracellular matrix (ECM) and in plasma. These ubiquitous proteins mediate cell attachment to the ECM by binding a variety of cell surface integrin proteins. In addition, they play key roles in cell migration, growth, and differentiation.

Table I: RetroNectin Supports High-Efficiency Gene Transfer[1]

Cell Type	Efficiency of Gene Transfer (%)
Human $CD34^+$ $CD38^-$ BMC[2]	95.5
Human PBMC[3]	91.2
TF-1	97.9
SupT1	97.3
Jurkat	80.1
K-562	90.4
HL-60	86.1
Monkey $CD34^+$ BMC	72.0
Monkey $CD4^+$ T-cell	85.0

1 Transductions were performed using the RetroNectin-Bound Virus (RBV) Method of transduction.
2 Bone marrow cells.
3 Peripheral blood mononuclear cells.

RetroNectin (CH-296) is a recombinant peptide, derived from several FN fragments that bind either viruses or cell surface proteins. RetroNectin has three functional domains: an RGDS motif-containing cell adhesion domain, which binds the cell surface integrin receptor VLA-5; a heparin-binding domain that binds many types of virus particles; and a CS-1 sequence that binds the VLA-4 cellular integrin receptor (Figure 1).

RetroNectin's Mechanism of Action

The multivalent nature of RetroNectin allows the simultaneous binding of cells and virus, bringing the two into close physical proximity (Figure 1). The hypothetical mechanism of action is that the colocalization of viruses and cells facilitates infection, resulting in higher frequencies of stable gene transfer.

A Protocol for Improved Retroviral Transduction

RetroNectin is used most effectively in the *RetroNectin-Bound Virus* (RBV) method of transduction, in which viruses are preloaded onto RetroNectin-coated plates, followed by the addition of target cells. Cells are infected as they come into contact with the RetroNectin/virus-coated substratum. The RBV method works well for all types of target cells, but it is especially useful for cells that are difficult to transduce, or if the infection requires a large amount of virus stock.

Remove Transduction Inhibitors and Improve Target Cell Viability

Inhibitors present in packaging cell supernatants can markedly reduce transduction efficiency, while other impurities or excess virus can affect the viability of sensitive cell types (3). In the RBV method of transduction, in which RetroNectin-coated plates are preloaded with virus, unbound virus particles and soluble inhibitors are washed from the plates prior to the addition of the cells to be infected. As a result,

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Improve Viral Transductions with RetroNectin® ...continued

target cells are transduced more efficiently and with less virus, while transduced cells demonstrate consistently better viability.

RetroNectin Increases Gene Transfer Efficiency

Takara researchers have found that RetroNectin-based protocols support highly efficient transduction in a variety of different cell types. For example, 90% of human CD34⁺ bone marrow cells; >70% of human and nonhuman primate CD4⁺ T lymphocytes; >80% of cells from human T lymphocyte cell lines, including K562, Jurkat etc.; and 90% of HT1080 cells were transduced using RetroNectin protocols. Transduction results for several other cell lines are shown in Table I.

Improved Transduction of Stem Cells and Jurkat Cells—Without Polybrene

RetroNectin-based protocols can be used to transduce human stem cells more efficiently than traditional methods that rely on either polybrene or protamine. For three different types of human stem cells, each expressing a different level of Pit-1 (the receptor for the GaLV Env protein; Table II), the RBV protocol yielded excellent transduction efficiencies in the range of 50–75% (Figure 2). In contrast, standard transduction protocols using either protamine or polybrene were much less effective.

For the difficult-to-transduce Jurkat T-cell line, the RBV protocol also works extremely well. Figure 3 shows results for Jurkat cells that were effectively transduced by either a retroviral or lentiviral vector. Following these infections, ZsGreen1-expressing transduced cells comprised 75% and 95% of the respective cell populations.

Table II: Surface Protein Phenotypes of Some Human Stem Cells

Gene	hCD34⁺	hMSC	hAD-SC
VLA-4[1]	+	–	+
VLA-5[1]	+	+	+
Pit-1[2]	0.36	1	0.31

1 VLA-4 and VLA-5 are integrins that bind RetroNectin.
2 Pit-1 is the cellular receptor for the GaLV Env protein. These values represent Pit-1 expression relative to that of hMSC.

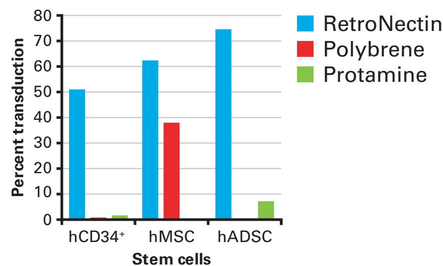

Figure 2. RetroNectin-mediated transduction outperforms standard methods of transduction. A GaLV-pseudotyped retrovirus carrying a fluorescent protein marker was used to transduce human hematopoietic (hCD34⁺), mesenchymal (hMSC), and adipose (hADSC) stem cells, all of which express different levels of Pit-1 (the GaLV receptor) (see Table II). In each case, the RBV method transduced the stem cells more efficiently than either the polybrene- or the protamine-based method.

Using RetroNectin Reagent and Precoated Culture Dishes

RetroNectin Reagent is used by coating it onto plates at a concentration of 20–100 µg/ml, which will cover the plates at 4–20 µg/cm². The 5 x 0.5 mg package contains enough RetroNectin to coat 10–60 dishes (3.5 cm diameter, 10 cm² surface area). RetroNectin Precoated Dishes are 3.5 cm in diameter and precoated at 40 µg/ml.

References

1. Hanenberg, H. *et al.* (1996) *Nature Med.* **2**(8):876–882.
2. Hanenberg, H. *et al.* (1997) *Hum. Gene Ther.* **8**(1):2193–2206.
3. Chono, H. *et al.* (2001) *J. Biochem.* **130**(3):331–334.

Product	Size	Cat. No.
RetroNectin Reagent		
	0.5 mg	T100A
	5 x 0.5 mg	T100B
RetroNectin Precoated Dishes		
	10 dishes	T110A

Notice to Purchaser

For international orders of Takara products, please refer to the Takara Bio website (**www.takara-bio.com**) to locate a distributor in your area.

Please see the RetroNectin® Reagent licensing statement on page 24.

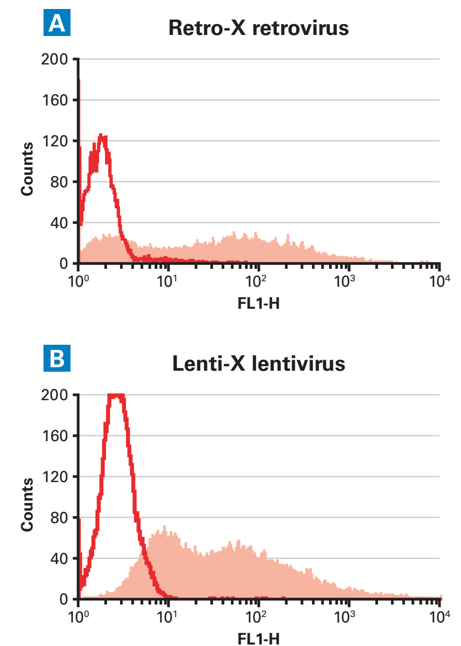

Figure 3. Effective retroviral and lentiviral transduction of Jurkat cells on RetroNectin-coated plates. Jurkat cells were transduced with either retrovirus (**Panel A**) or lentivirus (**Panel B**) by using serial RBV-based transductions. Each VSV-G-pseudotyped virus was engineered to express ZsGreen1. Viruses were bound to RetroNectin-coated plates for 4 hr followed by incubation with the cells for 24 hr. Following the first infection, the cells were collected and added to a second RetroNectin plate containing freshly bound virus. At 96 or 72 hr after the second round of retroviral or lentiviral infection, respectively, the cells were analyzed for fluorescent protein expression using flow cytometry. The transduced cells shown in **Panel A** were 75.5% positive with an MFI of 138; those shown in **Panel B** were 94.8% positive with an MFI of 120.