# FAQs: RetroNectin® Recombinant Human Fibronectin Fragment

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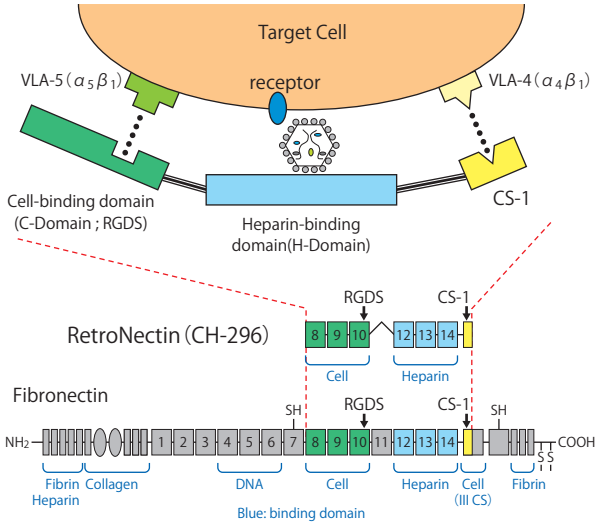
### Q1 What is Fibronectin?

**Answer 1:** Fibronectin is high-molecular weight (220-250 kD) component of the extracellular matrix. Fibronectin exists as a dimer and is involved in cell migration, morphology, growth, and adhesion.

### Q2 How does RetroNectin reagent co-localize viral particles and cells? Where do the virus particles bind?

**Answer 2:** RetroNectin is a ~63 kD protein (574 amino acids) that contains a central cell-binding domain (type III repeat 8, 9,10), a high affinity heparin-binding domain II (type III repeat 12, 13, 14), and CS1 site within the alternatively spliced IIICS region of human Fibronectin. RetroNectin reagent can enhance retroviral-mediated gene transfer into mammalian cells by co-localizing retrovirus particles and target cells. Co-localization is thought to be accomplished on the recombinant human Fibronectin CH-296 chimeric molecules by direct binding of retroviral particles to sequences in the heparin-binding domain II and cells via interaction with VLA-4 and/or VLA-5.



# FAQs: RetroNectin® Reagent, cont.

**Q3 What are Very Late Antigen-4 (VLA-4) and Very Late Antigen-5 (VLA-5)?**

**Answer 3:** VLA-4 and VLA-5 belong to the integrin superfamily and are also known as integrin α4β1 and integrin α5β1, respectively. Integrins are cell surface heterodimers that mediate the interaction between the extracellular matrix and intracellular cytoskeletal proteins.

**Q4 What kind of cells can RetroNectin reagent be used with?**

**Answer 4:** RetroNectin reagent is highly effective for cells that express integrin VLA-4 or VLA-5. These integrin molecules can interact with the CS1 site and the cell-binding domains of Fibronectin.

**Q5 Which cell types express VLA-4? Which cell types express VLA-5?**

**Answer 5:** VL-4-expressing cells include T cells, B cells, monocytes, NK cells, eosinophils, bone marrow monocytic cells, and lymphoid progenitors. Thymocytes, activated T-cells, and mast cell express VLA-5.

**Q6 Does RetroNectin reagent work for retroviruses packaged with different envelopes (Eco, Ampho, VSVG pseudo-type)?**

**Answer 6:** RetroNectin reagent works for both ecotropic and amphotropic retroviruses. Moreover, this product is useful for retroviruses with a GALV type envelope. RetroNectin-coated plates may also be effective for transfection using VSVG (Vesicular stomatitis virus G) pseudotyped retrovirus.

**Q7 Can RetroNectin reagent be used for introducing viral vectors other than retroviruses or for introducing DNA?**

**Answer 7:** RetroNectin reagent can be used to enhance lentiviral transduction. It is not known if RetroNectin is useful for introducing nucleic acids.

**Q8 How much can RetroNectin reagent increase transduction efficiency?**

**Answer 8:** When the viral supernatant infection method is used (cells and virus are mixed, then exposed to RetroNectin reagent), RetroNectin reagent increases transduction efficiency by 50-70% for human CD34+ cells and by 20-30% for mouse bone marrow mononuclear cells.

**Q9 How much cytotoxicity is typically observed when using RetroNectin reagent?**

**Answer 9:** Less toxicity has been observed with RetroNectin reagent compared to polybrene. For example, there is almost no effect on cellular growth when NIH3T3 cells are grown in medium containing 0.25 mg/ml of RetroNectin reagent.

**Q10 Can RetroNectin reagent be used in combination with polybrene?**

**Answer 10:** We recommend avoiding the simultaneous use of both reagents. Gene transfer efficiency decreases when polybrene is used concomitantly with RetroNectin reagent.

**Q11 What type of plate can I use with RetroNectin reagent?**

**Answer 11:** Non-coated tissue culture plates or dishes should be used for RetroNectin coating. Takara Bio has tested both coated and non-coated plate types and found that coated plates have only about 1/5 the efficiency of non-coated plates. We recommend using a 6-well BD-Falcon (# 351146) plate.

**Q12 What transduction efficiency can be obtained if coated tissue culture plates are used (even though this plate type is not recommended)?**

**Answer 12:** Experimental data indicate that transduction efficiency is above 30% when using RetroNectin-coated non-tissue culture treated plates, but only 1-3% when using RetroNectin-coated tissue culture treated plates. The latter is an example using a surface-treated plate. Although comprehensive data are not available regarding use of RetroNectin-coated plates which are also coated with other reagents (e.g., collagen), it is anticipated that the transfection efficiency will be lower whenever surface-treated plates are used.

# FAQs: RetroNectin® Reagent, cont.

However, if double coated plates are necessary, please test the following two coating methods: (1) coat with RetroNectin reagent, then collagen (or other substance), and (2) coat with collagen first, then RetroNectin reagent.

If transfection efficiencies are very low in both of the above cases, please try the following method:

Perform the transduction procedure using a RetroNectin-coated non-tissue culture treated plate as described in the user manual. After infection, incubate for 4 hours in a 37°C, 5% $CO_2$ incubator. Then collect the cells and transfer to the collagen (or other substance)-coated plate and incubate 2-3 days.

### Q13 How many times can RetroNectin reagent be frozen and thawed?

**Answer 13:** RetroNectin solution (1 mg/ml) can be frozen and thawed up to 5 times. Avoid additional freeze-thaw cycles, and be careful not to introduce contamination.

RetroNectin solution is stable for one year at -20°C or for approximately three weeks at 4°C.

### Q14 How long are pre-coated RetroNectin plates stable?

**Answer 14:** Pre-coated RetroNectin plates can be stored at 4°C and are stable for 1-2 months (although we recommend using them as soon as possible). Do not store pre-coated plates at -20°C.

### Q15 Does RetroNectin reagent have to be filter sterilized? If so, what type of filter should be used?

**Answer 15:** RetroNectin reagent is provided as a sterile solution. Therefore, filtration is not necessary.

If you choose to filter RetroNectin solution, we recommend using Millipore SLGV013SL or SLGV004SL filters.

### Q16 Does Takara Bio provide a clinical-grade RetroNectin reagent?

**Answer 16:** Yes. RetroNectin (GMP) grade (# T202) is manufactured as a quality-assured product according to guidelines for Good Manufacturing Practice (GMP) for Investigational Products and can be used for *ex vivo* clinical applications (limited to investigational use only).

### Q17 Can cell-culture bags, like X-fold bags, be coated with RetroNectin reagent?

**Answer 17:** Yes, X-FOLD bags can be coated with RetroNectin reagent. Below is a protocol that has been tested:

For an X-FOLD Bag (85 $cm^2$):

a) Coat the bag with RetroNectin reagent* overnight at 4°C or for 2 hours at room temperature.
* Use at least 9 ml of RetroNectin solution (20 µg/ml) per bag.

b) Wash with PBS (30 ml x 3 times).

c) Preload the Retrovirus (4-6 hours).

d) Wash with PBS (30 ml).

e) Add cells (1 x $10^4$ cells/$cm^2$).

f) Incubate at 37°C for 3 days under 5% $CO_2$.

NOTE: This method may not be applicable for other types/brands of cell-culture bags.

# FAQs: RetroNectin® Reagent, cont.

**Q18 Is there a way to improve transduction efficiency (e.g., 60%-70%)?**

**Answer 18:** Below are some tips on how to improve transduction using RetroNectin reagent. Even without these recommendations, RetroNectin reagent should give better transduction efficiencies than Fibronectin.

a) We recommend preparing retrovirus supernatant at a high titer ( $>10^6$ cfu/ml is desirable). To achieve transduction efficiencies of 60-70%, concentrate the retrovirus solution (e.g., measured titer = $1.15 \times 10^6$) more than 10-fold and use it for pre-loading.

b) Pre-load viral particles onto RetroNectin reagent-coated dishes at 200-250 µl/cm$^2$, and incubate for 4-5 hours at 32°C for best results.

c) Be careful not to let the virus-loaded plate dry after discarding the supernatant and washing with PBS. Add target cells in growth medium immediately after removing the supernatant. If the plate dries, the transduction efficiency will decrease remarkably.

**Q19 What procedure do you recommend for dissociating RetroNectin reagent-treated cells from plates?**

**Answer 19:** For strongly adherent cells, like fibroblasts, use trypsin prepared in EDTA/phosphate-buffered saline (PBS) (without $Ca^{2+}$ and $Mg^{2+}$) to dissociate cells.

For weakly adherent (or suspension) cells, use a 0.02% EDTA/PBS solution rather than trypsin to dissociate cells. Use trypsin to remove these cells only if the EDTA/PBS solution is unsuccessful; trypsin may damage these cells.

Sample procedure for weakly adherent cells:

a) Following transfection, transfer the supernatant from the plate to a centrifuge tube.

b) Wash the plate with PBS to recover non-adherent cells.

c) Dissociate adherent cells from the plate using Cell Dissociation Buffer (GIBCO; enzyme free, PBS-based) following the manufacturer's instructions.

d) Combine all obtained cells in one centrifuge tube, and centrifuge to recover the cell fraction.

e) Rinse cells with HBSS/Hepes twice by centrifugation, and suspend cells in HBSS/Hepes for further use.

**Q20 After cells are dissociated from a RetroNectin reagent-coated plate using either Trypsin or Cell Dissociation Buffer, are the cells likely to rebind to the same plate? If performing FACS in a 96-well format (high throughput), should the cells be transferred to a new plate?**

**Answer 20:** Cells dissociated by trypsin or Cell Dissociation Buffer from a RetroNectin reagent-coated plate will likely rebind to the same plate. We recommend transferring cells into another plate for FACS using a multi-channel pipette.

**Q21 After transduction of cells using RetroNectin reagent, what method do you recommend to resuspend the cells from the plate? I am using Jurkat cells and I want to stain the CD154 and CD69 cell surface markers and perform flow cytometry, and trypsin may degrade my cell surface markers.**

**Answer 21:** For cell lines such as HL60 cells and K562 cells, we have verified that cells can be easily collected by pipetting from the RetroNectin reagent-coated dish. However, for several other cell lines, it may be difficult to collect the cells by pipetting without trypsin treatment.

Resuspension of Jurkat cells has not been tested by Takara Bio. In general, if cells cannot be collected by pipetting, we recommend collecting cells by trypsin treatment 24 hours after transduction and transferring the collected cells into a fresh dish. Then culture the cells in suspension for ~2 days, and collect the suspended cells for flow cytometry.