Date Modified 04.05.2015

**CD34+ cells Lentivirus infection protocol on Retronectin.**

1. Thaw a vial of lin- CB or CD34+ cells at 37°C

- to 1 mL of thawed cells, immediately add 10 mL of IMDM/10%FBS medium, drop wise slowly while gently vortexing/shaking the tube.

2. Let stand at RT for 10 min

3. Spin 10 min 1200rpm, resuspend in 1ml Gene Transfer medium and count.

4. **Retronectin coating of the plate.**

* On the day of infection, coat the tissue culture treated multiwell plate with Retronectin. Calculate how many wells you need to coat.
* Dilute Retronectin stock (1000ug/ml) to the final concentration (10ug/ml) in sterile PBS-/-.

(for example add 5ul Retronectin to 0.5ml PBS. Mix gently by pipetting. **Filter!!!!)**

* Add 25-100ul of diluted Retronectin per well. Incubate for 2h at the room temperature (you can also precoat your cells a day earlier).
* Aspirate Retronectin. Block it with PBS/2%BSA for 20min. When you are ready with CD34+ cells aspirate PBS/2%BSA and plate the cells.

5. In each well of a 24 well suspension plate include:

-300,000 to 500,000 cells

-50-200 uL lentivirus (MOI between 5 and 100)

-top up to 400 uL with GT medium.

For 96 well plate scale accordingly

5. Leave at 37°C overnight, for 12-18 hours

|  |
| --- |
| **GENE TRANSFER MEDIUM (GT medium)**  X-VIVO 10 (BioWhittaker)  1%BSA (dilute from 10% stock)  P/S  L-Glu |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **RETROVIRAL GENE TRANSFER CYTOKINES (RGT)** | | | | |  |
|  | stock (ug/mL) | final conc (ng/mL) | for 1 mL (uL) | for 10 mL (uL) |  |
| IL-6 | 10 | 10 | 1 | 10 |  |
| SCF | 100 | 100 | 1 | 10 |  |
| Flt3 | 10 | 100 | 10 | 100 |  |
| G-CSF | 10 | 10 | 1 | 10 |  |
| TPO | 50 | 15 | 0.3 | 3 | + |
|  |  |  | 13.3 | 133 |  |
| Filter .2 µm | |  |  |  |  |
| Add 13.3 µl/mL in gene transfer medium | | |  |  |  |

RetroNectin Reagent is a recombinant human fibronectin fragment that contains three functional domains: the cell-binding domain (C-domain), the heparin-binding domain (H-domain), and the CS-1 sequence. RetroNectin Reagent enhances lentiviral- and retroviral-mediated gene transduction by aiding the co-localization of target cells and viral particles. Specifically, virus particles bind RetroNectin Reagent via interaction with the H-domain, and target cells bind mainly through the interaction of cell surface integrin receptors VLA-5 and/or VLA-4 with the fibronectin C-domain and CS-1 sites, respectively. By facilitating close physical proximity, RetroNectin Reagent can enhance viral-mediated gene transfer to target cells expressing integrin receptors VLA-4 and/or VLA-5.

<http://www.clontech.com/takara/IL/Products/Molecular_Biology/Gene_Transfer_and_Expression/xxclt_displayImage.jsp?imgCntId=11249&sitex=10032:22372:US>

