Introduction:

This SOP describes the procedure of transducing human T cells with retroviral supernatant.

For generation of retroviral supernatant turn to SOP-D-001.

Materials:

PBMC to transduce

Feeders	optional
Media:	AIMV/RPMI + 10% human serum + ABX + 300 IU/mL IL2
OKT3:	anti-CD3 (Miltenyi Biotec, stock 100 µg/ml)
Retroviral supernatant:	See SOP-D-001 for retroviral supernatant generation
rhIL-2	stock $6*10^6$ IU/ml
Retronectin	Takara #T100A/B (stock 1 mg/ml)
PBS/2%BSA	10g BSA (Sigma) in 500 ml PBS → filter through 0.22 µM filter
Non-tissue-culture coated plates:	24-wells
Tissue-culture coated plates:	24-wells or 6 wells

Day					
Day	**1**	**2**	**3**	**4**	**5**
SOP-D-001	Plate 293-GP **(see SOP-D-001)**	Transfect 293-GP; change media after 6 hrs **(see SOP-D-001)**		Harvest 48 hr sup **(see SOP-D-001)**	Harvest 72 hr sup (optional) **(see SOP-D-001)**
THIS SOP		Stimulate PBMC	Coat plates with retronectin	Transduce	Move cells off retronectin-coated plates

Methods:

Day 2: Stimulation of T Cells to be Transduced (Pick option A, B, or C)

A. Stimulation of bulk PBMC

- Thaw donor PBMC and count :_____
- Plate $7.5*10^6$ cells per 24-wells well in 2 ml media (50/50 media + 300 IU/mL IL2 + 50 ng/mL OKT3)

B. Stimulation of CD4 or CD8 depleted T cells

- Thaw donor PBMC and count :_____
- Perform CD4 or CD8 MACS-bead depletion (leaving other cells in PBMC intact) according to the MACS protocol.
- Plate $7.5*10^6$ cells per 24-wells well in 2 ml media (50/50 media + 300 IU/mL IL2 + 50 ng/mL OKT3)

C. Stimulation of CD4 or CD8 positively selected cells (requires feeders)

- Thaw donor PBMC and count.
- Perform CD4 or CD8 positive MACS-bead selection according to the MACS protocol.
- Thaw and irradiate feeder cells at 4000 RAD.
- Combine selected cells 1:10 with feeders ($3.75*10^6$ cells/mL feeders and $3.75*10^5$cells/mL CD4+ or CD8+ T cells) in 50/50 media + 300 IU/mL IL2 + 50 ng/mL OKT3.
- Plate $7.5*10^6$ cells per 24-wells well in 2 ml media (50/50 media + 300 IU/mL IL2 + 50 ng/mL OKT3)

Day 3: Retronectin coating of plates

- Coat non-tissue culture 6-well plates with retronectin at final concentration of 20 μg/ml in PBS
- Add 1.5ml retronectin/ well
- Wrap and incubate o/n at 4°C

Day 4 Retroviral transduction

- Run centrifuge at 2000xg at 32°C; it takes about 1 hr

- Aspirate retronectin solution from the 6-wells plate; or keep and store
- Block with 2 ml 2% BSA in PBS for 30 min at RT
- Thaw or harvest the retroviral supernatant (spin down for 10 minutes at 1000 RPM)
- Wash the 6-wells plate with 2 mL/well PBS, leaving the wash on the plate until just before the viral supernatant is added
- Aspirate the PBS and add **2 mL/well** viral supernatant (can also dilute supernatant 1:1 with DMEM and plate 1 mL/well)
- Wrap with plastic wrap and spin the plate at 2000xg for 2 hrs at 32^{o}C
- After 90 min, harvest, wash and count T cells to be transduced. Take into account untransduced wells, and control (GFP) transduced wells
- When the plates finish spinning, aspirate the viral supernatant from the plates
- Add **1-2 10^{6} T cells/well** in the AIM V media (300 IU/mL IL2) at the concentration 0.5e6/ml
- Wrap the plate, and spin for 10 min at 1500 RPM with the acceleration and brake set at 1
- Gently remove the plate from the centrifuge, check the cell density, and leave in the incubator o/n

Day 5: Remove from non-tissue cultured plates

- Transfer transduced cells off of retronectin, onto a tissue culture-coated 6-well plate. Consider combining every identical wells from the transduction plate into T25 or T75 Flask at concentration 0.5e6/ml
- Check GFP under the microscope.

Day 7: FACS analysis (day 3 post-transduction)

- Stain for mConstant region or another marker if and acquire cells on a flow cytometer
- Split cells if they are growing well and replenish with T cell media

Day 10: REP

- REP cells if needed