Retronectin Transduction Protocol

Dissolve Retronectin lyophilized powder in sterile water to a concentration of 1mg/ml by gentle swirling (do not vortex) and filtrate through 0.22µm filter (Millipore Millex GV). Store solution in aliquots at –20°C (can be stored for one year).

Coating:
Use only non-tissue culture-treated six-well plates (Becton Dickinson 351146, polystyrene) otherwise it won't work.

Dilute the 1mg/ml stock solution of Retronectin to a concentration of 25-50µg/ml in sterile PBS.

Dispense 2ml of the diluted Retronectin into each well and incubate the covered plate for 2 hours at room temperature (or at 4°C overnight).

Remove Retronectin solution and block each plate with 2 ml PBS (containing 2% BSA) for 30 minutes at RT.

Remove PBS (+2% BSA) and wash the wells once with 2 ml PBS.

(The coated plate can be stored at 4 °C for one week)

Remove the PBS and add 2ml of your virus supernatant in each well. Seal the 6 well plates with parafilm and centrifuge the plates for 2 hours with 2000xg at 32 °C.

Discard the virus supernatant (plate should not dry) and wash the wells with 2ml PBS.

(Optimal: add another 2 ml virus supernatant and centrifuge the plate for 2 hours with 2000xg at 32°C)

Add $1x10^5$ Cells /cm^2 in each well with growth medium and centrifuge for 30 min with 600xg at 32°C.

Incubate the cell for 2-days in the incubator (32-37°C).