

Day 1: Plate 293T

Coat 10cm dish with polylysine: Add 3-5ml, swirl to coat then remove.

Plate 4×10^6 293T cells per 10cm dish in D10 plus media. Incubate 37C overnight.

Day 2: Transfection

Remove media from plated 293T cells and add 5ml OPTIMEM media

For each 10cm plate mix 10ug Vector + 10ug pHIT60 + 2.5ug Eco Env + 1.5ml OPTIMEM

For 10 plates multiply by 10.25

=102.5ug Vector + 102.5ug pHIT60 + 25.6ug Eco + 15.4ml OPTIMEM.

Pass through 0.22um Filter and let sit 5 min

For each 10cm plate mix 36ul lipofectamine with 1.5ml OPTIMEM = 369ul lipofectamine+ 15.4ml OPTIMEM

Add DNA mixture to lipofectamine mixture slowly, incubate room temp 20 min, inverting several times

Remove 3ml of OPTIMEM media from 293 plates (leave 2ml) and add 3ml of DNA/lipofectamine mixture to each plate. Add it dropwise and swirl the plate to mix. Incubate 3-4 hours at 37C

After 3-4 hours add 5ml D10Plus media and incubate overnight

Day 3: Sodium Butyrate Induction

Remove Media from plates and add 8ml of D10 Plus media + 10mM Sodium Butyrate. Incubate 8 hours

After 8 hours, remove media and add 10ml fresh D10 Plus media. Incubate 37 overnight

Day 4: First Viral Supernatant Collection

Harvest 10ml of supernatant from each plate 21-24 hours after last media change. Filter thru a 0.45um filter. Use immediately or store long term at -80C. Can store at 4C short term (~24-36 hours). Longer storage at 4C results in lower titer virus.

Replace with 10ml of fresh D10 Plus media. Incubate 37 overnight

Day 5: Second Viral Supernatant Collection

Retrovirus Production Muschen Lab 2010

Harvest 10ml of supernatant from each plate 21-24 hours after last media change. Filter thru a 0.45um filter. This is the last collection, the cells can now be discarded.

Reagents:

10cm tissue culture treated dishes

Sigma Poly-L-Lysine Cat#P4707

D10 Plus Media: High Glucose DMEM+10%FBS+1x PenStrep+25mM HEPES+1mM Sodium Pyruvate+0.1mM Non-essential Amino Acids, 6mM Glutamine

Invitrogen DMEM High Glucose + Glutamax (glutamine) + Sodium Pyruvate
Cat#10569-010

Invitrogen OPTIMEM media Cat#31985-070

Invitrogen MEM NEAA 100X Cat#11140

Invitrogen HEPES 1M Cat#15630

Invitrogen Lipofectamine 2000 Reagent Cat#11668-019

0.45 micron and 0.22 micron Filters

Retroviral Packaging Plasmid pHIT60 (Ref Soneoka et al Nucleic Acids Research 1995 23:628-633)

Ecotropic Envelope Plasmid

0.5 M Sodium Butyrate Stock Solution (sterile filtered for tissue culture)