

Establishing HaCaT (human keratinocyte cell line) stable line

Culture medium: DMEM+10% FBS

Day 1

Plate HaCaT in 10 cm dish (~50% confluent ← % depends on cell type)

Day 2

Transfection

Remove medium

Add new 4 mL DMEM+10% FBS to above culture

In 1.5 ml tube, mix below

250 μ L serum free DMEM

25 μ L PEI Max

5-10 μ g Plasmid (Hyg-r containing vector)

15 min, r.t.

Add to cells, 37°C

Day 3

Add 4mL DMEM+10% FBS to cells

Day 4

Wash w/ PBS

Add 1xTE, resusp w/

8mL DMEM+10% FBS containing ~1 mg/mL Hyg

Day 9

Change media to 0.8 or 0.4 mg/mL Hyg

Day 16

Wash w/ PBS

Add 1xTE, resusp w/

8 mL DMEM+10% FBS containing 0.8 or 0.4 mg/mL Hyg

Day 22

Wash w/ PBS

Add 1xTE, resusp w/

8mL DMEM+10% FBS containing 0.8 or 0.4 mg/mL Hyg

Check expression

Note: HaCaT cell may do CMV promoter silencing