

PROMOTER CONSTRUCTS: SAMPLE PROTOCOL FOR K562 SUSPENSION CELLS

DAY 1

Goal: Prepare reporter constructs, seed and transfect cells simultaneously

Prepare constructs

- Thaw Switchgear constructs (plasmid DNA) at room temperature.
- Centrifuge the tubes or plates to remove condensation from lid.

Count cells

- Ensure you are using low passage K562 cells in antibiotic free media with greater than 90% viability
- Count cells to determine if you have enough cells and adjust the volume according to the number of desired transfections (see Table 1)

Table 1

Number of wells in plate	Volume of complete, antibiotic-free media	Number of cells/well
96	80 μ l	25,000

- Reserve the appropriate number of cells for later for combining with the transfection mixture later

Transfections

- Combine the reagents in Table 2 for each transfection. Conduct at least three replicates per transfection.
- Easy tip: Make a master mix of transfection reagent + OptiMEM add to each construct (add transfection reagent to OptiMEM without touching sides of tube). For each construct, make a master mix of DNA, transfection reagent, OptiMEM for desired number of replicates (make at least 1.5 extra aliquots to account for pipetting error and evaporation). See Appendix for the Experimental Design Example.

Table 2

Component	Per well (96-well format)
Opti-MEM (serum free media)	16.57 μ L
Switchgear plasmid DNA construct (30ng/ μ L)	3.33 μ L
PLUS™ Reagent	0.1 μL
TOTAL	19.6μL

- Mix DNA, PLUS™ reagent and OptiMEM combination well. Let sit at room temperature for 5-15 minutes.
- For each well transfected, add 0.4 μ L Lipofectamine LTX to the DNA, PLUS™ reagent and OptiMem combination
- Mix gently and incubate for 25 minutes at room temperature
- For each transfection, add 80 μ L of appropriately diluted cells in complete media (Table 1) to the incubated transfection mixture

- Easy tip: if performing three or more replicates, it may be easier to add the appropriately volume of dilute cells to a deep well block and then transfer the transfection mixture to the block
- Mix cell/transfection mixture well
- Aliquot 100 μ L of cells plus transfection mixture into a 96-well assay plate
- Shake plate gently, cover with lid or breathable sealing tape
- Put back in incubator for 24-48 hours (+/- stimulus of interest)

Cells that transfect poorly may require the use of more DNA and/or longer incubation times. We recommend optimizing conditions for each cell line before beginning large-scale experiments.

DAY 3

Goal: Measure luciferase activity

- Remove plate from incubator and bring to room temperature
- Add 100 μ L (96-well format) Promega Steady-Glo Luciferase Assay Reagent, cover with lid or foil tape, and incubate for 15 minutes in a dark area.
- Read in a plate luminometer – 2 seconds per well

EXAMPLE CATALOG NUMBERS

Item	Vendor	Catalog Number
White Tissue Culture Plates (96-well solid bottom)	Greiner Bio-One	655083
Clear Tissue Culture Plates (96-well)	VWR	353072
White Tissue Culture Plates (384-well solid bottom)	Greiner Bio-One	781080
Clear Tissue Culture Plates (384-well)	VWR	781186?
Lipofectamine LTX and Plus Reagent	Invitrogen	15338-100
Opti-MEM	Invitrogen	31985-070
Steady-Glo Luciferase Assay Reagent	Promega	E2510, E2520
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Sealing Tape	E&K Scientific	T896100-S
	VWR	47749-926
Plate Luminometer	Molecular Devices	LMaxII-384