LiGHTSW!TCH
Luciferase Assay System

Protocol

GoClone Reporter Constructs: Sample Protocol for K562 Suspension Cells

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The LightSwitch Luciferase Assay System is a fully optimized reporter system that includes a transfection reagent (FuGENE HD), GoClone constructs utilizing the RenSP luciferase gene, and LightSwitch Luciferase Assay Reagents. Use all components of the LightSwitch System as recommended to obtain optimal reporter assay results.

DAY 1

Goal: Prepare reporter constructs, seed, and transfect cells simultaneously

Prepare GoClone Constructs

1. Thaw GoClone constructs (plasmid DNA) at room temperature.
2. 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.

3. Count cells to determine if you have enough cells and adjust the volume according to the number of desired transfections (see Table 1).

▶ Reserve the appropriate number of cells for combining with the transfection mixture.

Table 1

Number of wells in plate	Volume of antibiotic-free media per well	Number of cells per well
96	80 µL	25,000

__Tip:__ Make master mix #1 of transfection reagent + OptiMEM and add to each GoClone construct (add transfection reagent to OptiMEM without touching sides of tube). For each construct, make a master mix #2 of DNA, and master mix #1 for desired number of replicate transfections (make at least 1.5 extra aliquots to account for pipetting error and evaporation).

Transfections

4. Combine the reagents in Table 2 for each transfection. Conduct at least three replicates transfections per construct for each condition.
5. Mix DNA, PLUS™ reagent (Invitrogen) and OptiMEM combination well. Let sit at room temperature for 5-15 minutes.
6. Add 0.4 µL Lipofectamine LTX (Invitrogen) to 19.6 µL of DNA, PLUS™ reagent and OptiMEM combination for each transfection replicate.

Table 2

Component	Per well (96-well format)
Opti-MEM (serum free media)	16.08 µL
GoClone plasmid DNA construct (30ng/µL)	3.34 µL
PLUS™ Reagent	0.18 µL
TOTAL	**19.6 µL**

Tip: If performing three or more replicate transfections, it may be easier to add the volume of cells to a deep well block and then transfer the transfection mixture to the block.

7. Mix gently and incubate for 25 minutes at room temperature.
8. For each transfection replicate, add 80 µL of appropriately diluted cells in complete media (Table 1) to the 20µL incubated transfection mixture
9. Mix cell/transfection mixture well.
10. Aliquot 100µL of cells plus transfection mixture into one well of a white 96-well TC plate.
11. Shake plate gently, cover with lid or breathable sealing tape.
12. 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 with LightSwitch Assay Reagents

Note: Luciferase assays may be conducted immediately or the plates may be frozen at -80˚C (freezing generally increases cell lysis and luciferase signal). If using frozen plates, thaw and bring to room temperature before assaying.

1. Remove plate from incubator and bring to room temperature.
2. Prepare LightSwitch Assay Reagents (for LS010 kit, protocols of other kit sizes may be found online):
   A. Reconstitute 100X Substrate by adding 100µL of Substrate Solvent to tube of lyophilized Assay Substrate. *Protect from light and minimize time at room temperature. 100X Substrate may be stored at -20C for 2-3 weeks. For best results, use freshly reconstituted substrate.*
   B. Prepare Assay Solution by thawing 10mL bottle of Assay Buffer in room temperature water bath and add 100µL of reconstituted 100X Substrate prior to use. *For best results, avoid additional freeze-thaw cycles. To thaw re-frozen buffer, incubate in a warm (37C) water bath for at least 1 hour and mix well to ensure that all components go back into solution.*
3. Use a multi-channel pipettor to add 100µL LightSwitch Assay Solution (buffer+substrate) directly to each sample well in a white 96-well plate.
   If cells were grown in another plate or flask format, transfer samples to a white 96-well plate in 100µL total volume (media or PBS).
4. Cover plate, protect from light, and incubate for 30 minutes at room temperature.
   If assaying more than one plate, stagger addition of assay solution so that each plate incubates for 30 minutes before reading.
5. Read each well for 2 seconds in a plate luminometer (SpectraMax L or equivalent).

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
LightSwitch Luciferase Assay Reagent	SwitchGear	LS010
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Sealing Tape	E&K Scientific	T896100-S
	VWR	47749-926
Plate Luminometer	Molecular Devices	SpectraMax L