LiGHTSW!TCH
Luciferase Assay System

Protocol

LightSwitch Transfection Optimization Kit™

LightSwitch Transfection Optimization Kit™
Instructions for use in 96-well format, 4 day protocol

OVERVIEW

The LightSwitch Transfection Optimization Kit includes the key reagents and protocols needed for optimizing transfection conditions for your cell line or condition of interest. The kit utilizes FuGENE® HD, one of the most robust transfection reagents available. Our extensive experience with transfection and reporter assays have identified 3 key variables in successfully transfecting a variety of different cell lines:

1. FuGENE to plasmid ratio — 3:1 or 6:1 (nL Fugene : ng plasmid)
2. Cells seeded per well — 5,000 or 15,000 cells per well (96 well format)
3. Duration of transfection — 24 or 48 hours

PROTOCOL SUMMARY

Day 1: Seed cells
Day 2: Transfect
Day 3: Freeze 24hr plate
Day 4: Freeze 48hr plate, read plates

Qty	Description	Storage
1 tube	Positive Control GoClone™ construct	-20°C for 6 months
1 tube	Negative Control GoClone™ construct	-20°C for 6 months
200 uL	FuGENE® HD Reagent	4°C for 6 months
100 assays	LightSwitch Assay System™	-20°C for 6 months

Additional materials needed:

Item	Suggested vendor	Suggested catalog number
Opti-MEM	Invitrogen	31985-070
White TC Plates (96-well)	VWR	82050-736
Clear TC Plates (96-well)	VWR	353072
Foil Plate Sealing Tape	E&K Scientific	T592100
Breathable Plate Tape	E&K Scientific	T896100-S
Plate Luminometer	Molecular Devices	Spectra Max L

DAY 1 (morning): Seed cells

1. Trypsinize and count cells grown to confluence in T75 flask
You will need at least ~1,500,000 cells for this protocol. Trypsinize with 1mL 0.25% Trypsin for a T75 flask and resuspend in 10mL culture media. We recommend counting twice to ensure an accurate cell count.

2. Dilute cells to 150,000 cells/mL in a total volume of 15mL of fresh culture media in a 50mL conical tube, label as "Tube A"

3. Label a separate 50mL conical tube as "Tube B", create a more dilute aliquot of the cells by adding 5mL of cells from Tube A to 10mL of fresh media for a final concentration of 50,000 cells/mL

4. Label two 96-well white TC plates "24hr" and "48hr". Seed 100uL of cells from tubes A and B according to the plate maps below
To assess confluence, also seed 100uL in at least 6 wells of a clear 96-well TC plate with cells from both tubes A and B.

5. Cover the plates with lid and place in TC incubator

24hr

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			A	A	A			A	A	A		
C			A	A	A			A	A	A		
D												
E												
F			B	B	B			B	B	B		
G			B	B	B			B	B	B		
H												

48hr

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			A	A	A			A	A	A		
C			A	A	A			A	A	A		
D												
E												
F			B	B	B			B	B	B		
G			B	B	B			B	B	B		
H												

DAY 2: Transfect

1. 24 hours after seeding, set up 4 separate transfection reactions according to the tables below:

1. **PC3** = Positive control, 3:1 FuGENE to plasmid ratio
2 **PC6** = Positive control, 6:1 FuGENE to plasmid ratio
3 **NC3** = Negative control, 3:1 FuGENE to plasmid ratio
4 **NC6** = Negative control, 6:1 FuGENE to plasmid ratio

Set up the reactions in 1.5mL eppy tubes in a tissue culture hood. Bring FuGENE HD to room temperature and mix by swirling the vial. First, add pre-warmed Opti-MEM media to each tube, followed by the plasmid DNA, followed by FuGENE and mix well. Add FuGENE directly to the Opti-MEM without touching the sides of the tube.

Positive Control, 3:1 — "PC3"

Component	Volume
Opti-MEM	63.6µL
Positive Control GoClone (30ng/µL)	33.4µL
FuGENE HD	3.0µL
TOTAL	100µL

Positive Control, 6:1 — "PC6"

Component	Volume
Opti-MEM	60.6µL
Positive Control GoClone (30ng/µL)	33.4µL
FuGENE HD	6.0µL
TOTAL	100µL

Negative Control, 3:1 — "NC3"

Component	Volume
Opti-MEM	63.6µL
Negative Control GoClone (30ng/µL)	33.4µL
FuGENE HD	3.0µL
TOTAL	100µL

Negative Control, 6:1 — "NC6"

Component	Volume
Opti-MEM	60.6µL
Negative Control GoClone (30ng/µL)	33.4µL
FuGENE HD	6.0µL
TOTAL	100µL

2. Mix transfection reactions well by pipetting up and down and incubate at room temperature for 30 minutes

3. Transfect cells according to the plate maps below by gently pipetting 5uL of the transfection reaction onto the top of the cell culture media

Each of the four reaction mixes will be transfected in triplicate (a,b,c), in both cell densities, in the 24hr and 48hr plate. 5uL of the transfection mix contains 100ng of plasmid DNA. Conduct transfections in a tissue culture hood. A 20uL repeat pipetter may be used to pipette three transfections at a time.

24hr

	1	2	3	4	5	6	7	8	9	10	11	12	
A			(tfx in triplicate)					(tfx in triplicate)					
B		(3:1)	PC3a	PC3b	PC3c			NC3a	NC3b	NC3c			(15K cells/well)
C		(6:1)	PC6a	PC6b	PC6c			NC6a	NC6b	NC6c			(15K cells/well)
D													
E			(tfx in triplicate)					(tfx in triplicate)					
F		(3:1)	PC3a	PC3b	PC3c			NC3a	NC3b	NC3c			(5K cells/well)
G		(6:1)	PC6a	PC6b	PC6c			NC6a	NC6b	NC6c			(5K cells/well)
H													

48hr

	1	2	3	4	5	6	7	8	9	10	11	12	
A			(tfx in triplicate)					(tfx in triplicate)					
B		(3:1)	PC3a	PC3b	PC3c			NC3a	NC3b	NC3c			(15K cells/well)
C		(6:1)	PC6a	PC6b	PC6c			NC6a	NC6b	NC6c			(15K cells/well)
D													
E			(tfx in triplicate)					(tfx in triplicate)					
F		(3:1)	PC3a	PC3b	PC3c			NC3a	NC3b	NC3c			(5K cells/well)
G		(6:1)	PC6a	PC6b	PC6c			NC6a	NC6b	NC6c			(5K cells/well)
H													

DAY 3 : Freeze 24hr plate

1. 24 hours post-transfection, remove the 24hr plate from the tissue culture incubator, remove lid, cover with foil tape, and immediately freeze at -80 C

DAY 4: Freeze 48hr plate

1. 48 hours post-transfection, remove the 48hr plate from the tissue culture incubator, remove lid, cover with foil tape, and immediately freeze at -80 C

LIGHTSWITCH LUCIFERASE ASSAYS

After both plates have been frozen for at least 6 hours, thaw and bring to room temperature before assaying luciferase activity.

LightSwitch Reagent Preparation:

1. Reconstitute 100X Substrate
Add 100uL Substrate Solvent to tube of 100X Substrate. Dissolve completely and mix well.
Protect from light and minimize time at room temperature. 100X substrate may be stored at -20C and protected from light for 2-3 weeks. For best results, use freshly reconstituted substrate.

2. Prepare Assay Solution
Thaw 10mL bottle of Assay Buffer in room temperature water bath and add 100 uL of reconstituted 100X Substrate just prior to use. Mix well.
Prepare Assay Solution (buffer + substrate mix) fresh for each use and use within 2-3 hours. To assay fewer wells, make up only what you need and store remaining substrate and buffer separately at -20C.

LightSwitch Assays:

1. Use a multi-channel pipettor to add 100uL Assay Solution (buffer+substrate) directly to each sample well in a white 96-well plate
Bring sample plates to room temperature. In general, cells may be assayed in white 96-well plates by thawing at room temperature for 45 minutes before assaying.

2. 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.

3. Read each well for 2 seconds in a plate luminometer (Spectra Max L or equivalent)

Data Analysis

1. Calculate the signal to noise ratio for each of the 8 conditions
Signal to noise =
(average of the 3 positives controls) / (average of the 3 negative controls)

2. Calculate the standard deviation of the 3 positive control replicates for each of the 8 conditions

3. Determine which condition gives the maximum signal to noise ratio with the least variation between replicate transfections