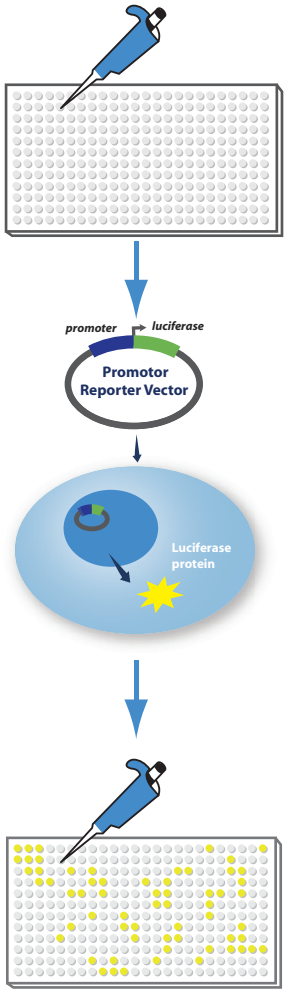
**LiGHTSW!TCH**
**Luciferase Assay System**

# Protocol

## GoClone Reporter Constructs: Sample Protocol for Adherent Cells

# LightSwitch Luciferase Assay System

## GoClone Reporter Assay Workflow




**Step 1:** Seed cells in plate format.

**Step 2:** Transfect SwitchGear GoClone reporter constructs into the cells using FuGENE HD. Apply stimulus of interest.

**Step 3:** Add LightSwitch Luciferase Assay Reagent and read on luminometer.

All SwitchGear reporter constructs utilize RenSP, a destabilized form of a novel luciferase developed by SwitchGear Genomics for its reporter gene assays. The optimized luciferase protein has a half-life of ~1 hour enabling a detailed analysis of kinetic responses with a highly robust signal.

## GETTING STARTED

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

- Use low passage number cells (highly passaged cells generally yield noisy data).
- Optimize the transfection protocol for your specific cell line and experimental design using the LightSwitch Transfection Optimization Kit (TFXOPT).

*Tip: LightSwitch assays have been optimized with HT1080 cells which are recommended for this protocol. Use standard media recommended by ATCC. Set up enough replicate transfections for control and treatment conditions.*

Important variables to test include:

| | |
|---|---|
| 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 hrs |

## DAY 1

**Goal:** Seed cells to yield 40-80% confluence after 24 hours

1. Seed the appropriate number of cells for the assay in white 96 or 384-well TC plates (see Table 1).
2. Seed additional wells in a clear TC plate in parallel for assessing confluence.

**Table 1**

| Number of wells in plate | Total volume for cell seeding per well | Number of cells/well that yields target confluency of 40-80% |
|---|---|---|
| 96 | 100µl | 5,000-15,000 |
| 384 | 50µL | 2,500-10,000 |

*Tip: Make Master Mix 1 containing enough FuGENE HD and OptiMEM (in the appropriate ratios) for addition to all construct wells. (Add FuGENE HD to OptiMEM without touching the sides of the tube.) Then, add an aliquot of Master Mix 1 to the DNA for each unique construct yielding Master Mix 2 (FuGENE HD + OptiMEM + DNA for X replicate transfections). For each Master Mix step, make some extra volume to account for pipetting error and evaporation. See Appendix for the Experimental Design Example.*

# DAY 2

**Goal:** Prepare GoClone reporter constructs and transfect into the seeded cells

## Prepare constructs and reagents

1. Thaw GoClone constructs (plasmid DNA) at room temperature. Mix well.
2. Centrifuge the tubes or plates of DNA to remove condensation from lid.
3. Bring FuGENE HD transfection reagent to room temperature, mix well.
4. Pre-warm Opti-MEM media in a 37˚C water bath.

## Transfections

5. Combine the reagents in Table 2 for each transfection. Conduct at least three replicate transfections per construct in each condition (e.g. untreated and treated). Volumes listed in the table are for a single replicate transfection in a single well. We recommend at least 50ng of GoClone plasmid DNA per well for 96-well experiments and 30ng of GoClone plasmid DNA per well for 384-well experiments.

**Table 2**

| LightSwitch Assay Component | Per well (96-well format) | Per well (384-well format) |
|---|---|---|
| FuGENE HD* Transfection Reagent | 0.15µL | 0.09µL |
| Opti-MEM (serum free media) | 3.18µL | 1.91µL |
| GoClone plasmid DNA construct (30ng/µL) | 1.67µL | 1.00µL |
| **TOTAL** | **5.00µL** | **3.00µL** |

* The FuGENE HD to plasmid DNA ratio shown in this example is 3:1 (150nL FuGENE HD to 50ng plasmid DNA)

6. Mix DNA, FuGENE HD, OptiMEM combination well. Let sit at room temperature for 30 minutes.
7. Gently drip 5µL or 3µL (96- or 384-well formats) onto seeded cells.
8. Shake plate gently, cover with lid or breathable sealing tape and return to incubator
9. Incubate for 24 to 48 hours before assaying.
10. Alternatively, incubate cells with transfection mix for 16 to 24 hours before changing conditions or applying a stimulus.

# 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).

# APPENDIX

## Experimental Design Example : transfection set-up for induction tests in 96-well plate format (triplicate transfections for each construct in each condition)

**24 constructs (experimental + controls), 3 conditions, 3 replicate transfections per condition**

### Step 1: Calculations (including extra for pipetting error and evaporation)

**Transfection mix calculations**

| | Per replicate well (96-well format) | Per construct (9 replicates + 1.5 extra) x 10.5 |
|---|---|---|
| FuGENE HD | 0.15µL | 1.57µL |
| Opti-MEM (serum free media) | 3.18µL | 33.39µL |
| GoClone plasmid DNA construct (30ng/µL) | 1.67µL | 17.54µL |
| **TOTAL** | **5.00µL** | **52.5µL** |

**Step 2:** Make Master Mix #1 – OptiMEM + FuGENE HD mix that will be added to each DNA aliquot

| | Per construct (9 replicates + 1.5 extra) | Master Mix #1 (24 constructs + 10% extra) x 26.4 |
|---|---|---|
| FuGENE HD | 1.57µL | 41.45µL |
| Opti-MEM (serum free media) | 33.39µL | 881.50µL |
| GoClone plasmid DNA construct (30ng/µL) | 17.54µL | Unique to each |
| **TOTAL** | **52.5µL** | **922.95µL** |

**Step 3:** Make Master Mix #2 = Master Mix #1 + DNA for all replicates of one construct

| | Master Mix #2 Per construct (9 replicates + 1.5 extra) |
|---|---|
| Master Mix #1 | 34.96µL |
| GoClone plasmid DNA construct (30ng/µL) | 17.54µL |
| **TOTAL** | **52.5µL** |

**Step 4:** Incubate transfection mixes at room temperature for 30 minutes

**Step 5:** For each GoClone construct, gently drip 5µL of Master Mix #2 onto each of the 9 replicate wells

**Example Catalog Numbers**

| Item | Vendor | Catalog Number |
|---|---|---|
| White Tissue Culture Plates (96-well solid bottom) | VWR | 82050-736 |
| Clear Tissue Culture Plates (96-well) | VWR | 353072 |
| White Tissue Culture Plates (384-well solid bottom) | VWR | 82051-278 |
| Clear Tissue Culture Plates (384-well) | VWR | 781186 |
| 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 |
| Plate Luminometer | Molecular Devices | SpectraMax L |
| FuGENE HD Transfection Reagent | SwitchGear | F500 |
| ACTB promoter positive control reporter construct | SwitchGear | S717678 |
| R01 negative control reporter construct | SwitchGear | S790001 |