LightSwitch™ Dual Assay System

DA010 (100 assays)

DESCRIPTION

The LightSwitch Dual Assay System is used to normalize for variation between transfection replicates in cell lines that are particularly difficult to transfect by utilizing a co-transfection control. Please read the **Do I Need a Co-transfection Control?** technical note to help determine whether your project needs a co-transfection control. Please note that the use of a co-transfection control is not sufficient for normalizing between conditions, treatments, or cell lines. For normalizing between experimental conditions, use LightSwitch promoter or 3'UTR control constructs.

All LightSwitch promoter and 3'UTR reporter constructs utilize RenSP, a novel luciferase developed by SwitchGear Genomics for 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. RenSP reporter signal is detected using LightSwitch Luciferase Assay Reagents. The Cypridina TK control construct (pTK-CLuc Vector) contains the Cypridina luciferase reporter gene driven by an HSV-TK constitutive promoter. Its signal is detected using the BioLux® Cypridina Luciferase Assay Kit developed by New England Biolabs.

The Cypridina TK control construct and LightSwitch GoClone experimental constructs contain different luciferase genes that use different substrates, so there is no cross-reaction between the two reporter genes and their substrates. As an added advantage, the Cypridina reporter protein is secreted into the culture media while the RenSP reporter protein remains within the cell. Normalizing the LightSwitch signal using the Cypridina reporter signal may decrease variation between transfection replicates in difficult cell lines.

See the **LightSwitch Luciferase Assay System Manual** and **BioLux® Cypridina Luciferase Assay Kit Manual** for more details.

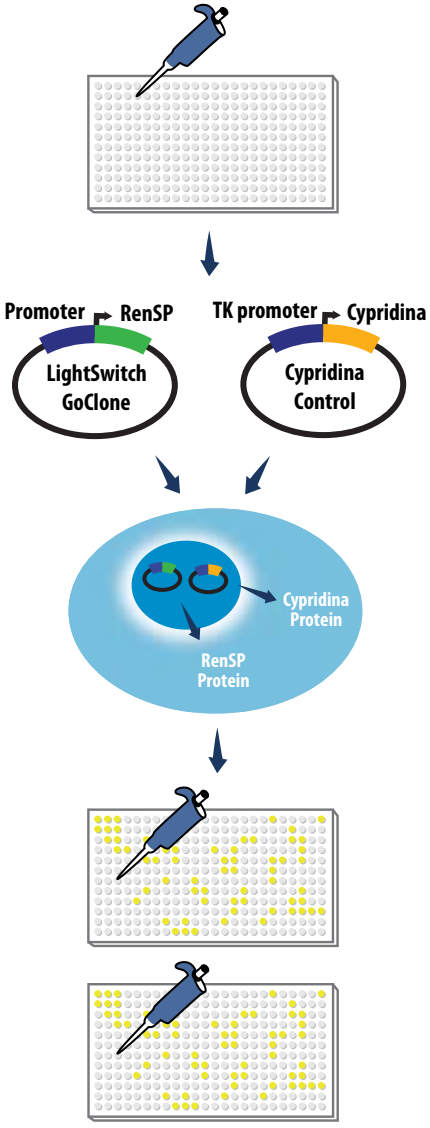
Components & Storage (100 assays)

1 tube	LightSwitch Assay Substrate (100X)	-20C for 6 months
150 uL	LightSwitch Substrate Solvent	room temp
10 mL	LightSwitch Assay Buffer	-20C for 6 months
1 tube	BioLux Cypridina Luciferase Substrate	-20C for 6 months
500 uL	BioLux Cypridina Luciferase Substrate Solvent	room temp
5 mL	BioLux Cypridina Luciferase Assay Buffer (1X)	-20C for 6 months

! Additional SwitchGear Product Needed:

Cypridina TK Control Construct (Cat # SN0322S)

(Cypridina luciferase gene regulated by the HSV-TK constitutive promoter)



LightSwitch Dual Assay Workflow

Step 1: Seed cells in plate format.

Step 2: Co-transfect a SwitchGear GoClone Construct with the Cypridina TK control construct into cells using FuGENE HD. Apply stimulus of interest.

Step 3: Transfer an aliquot of media to secondary assay plate.

Step 4: Add LightSwitch Luciferase Assay Reagent to primary assay plate and read on luminometer.

Step 5: Add BioLux Assay Reagent to secondary assay plate and read on luminometer.

GETTING STARTED

- 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 SwitchGear LightSwitch Transfection Optimization Kit (TFXOPT).
  - Important variables to optimize for your transfection:
    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
    4. Amount of supernatant removed for secondary assay

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 per well	Number of cells per well (confluency of 40-80%)
96	100µl	5,000-15,000
384	50ul	2,500-10,000

DAY 2

Goal: Prepare GoClone reporter constructs and Cypridina TK control construct and transfect into the seeded cells

Prepare constructs and reagents

1. Thaw GoClone constructs (plasmid DNA) and Cypridina TK control construct DNA at room temperature and mix well.
2. Centrifuge the tubes or plates of DNA to remove condensation from lids.
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 and 10ng of Cypridina TK control construct DNA per well for 96-well experiments.

Co-transfection Assay Component	Per well (96-well)	Per well (384-well)
FuGENE HD* Transfection Reagent	0.18µl	0.12µl
Opti-MEM (serum free media)	2.15µl	1.38µl
GoClone construct (30ng/µl)	1.67µl	1.00µl
Cypridina control construct (10ng/µl)	1.00µl	0.5µl
TOTAL	**5.00µl**	**3.00µl**

* The FuGENE HD to plasmid ratio shown in this example is 3:1 (180nl FuGENE HD to 60ng total 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 LightSwitch GoClone 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. Transfer 20µl supernatant to a second white 96-well plate and either set plate aside or freeze at -80C.
3. Prepare LightSwitch Assay Reagents
   A. Reconstitute 100X Substrate by adding 100µL of Substrate Solvent to tube of lyophilized Assay Substrate. *Protect from light and minimize time at room tem¬perature. 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.*

4. 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).*
5. 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.*
6. Read each well for 2 seconds in a plate luminometer (SpectraMax L or equivalent).

Goal: Measure Cypridina control construct luciferase activity with BioLux Assay Reagents

1. Thaw BioLux Assay Buffer (1X) completely to room temperature (protect from light) and mix well before use.
2. If using frozen supernatant, allow plate to thaw completely and bring to room temperature.
3. Prepare BioLux Assay Solution

   A. Add 0.5mL of absolute ethanol (not included) to the BioLux Substrate Solvent and mix well.

   B. Carefully lift the rubber cap of the lyophilized BioLux Substrate vial and add 60µL of the ethanol-solvent mixture to the lyophilized BioLux Substrate.

   C. Gently mix to dissolve the substrate (do not vortex, do not create bubbles by pipeting) and incubate at room temperature for 30 minutes (protect from light). Note: Be sure to dissolve any residual lyophilized powder on the rubber cap.

   D. Aliquot the reconstituted substrate (100x solution) and store at -80˚C if desired.

   E. Prepare BioLux Assay Solution by adding 50uL of reconstituted substrate to 5mL of BioLux Assay Buffer.
4. Add 50µL of the BioLux assay solution to each well of the assay plate that contains a Cypridina supernatant aliquot.
5. 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.*
6. Read each well for 2 seconds in a plate luminometer (SpectraMax L or equivalent).

Goal: Conduct normalization calculations

Compare well-to-well variation between LightSwitch Reagent only data and the data created using the Cypridina co-transfection control normalization to determine whether the co-transfection control will be needed in subsequent experiments. To determine whether the co-transfection decreases well-to-well variation, divide the raw signal from the LightSwitch reagent data by the raw signal from the corresponding BioLux data. Repeat the data analysis on these new values that account for differences in the Cypridina signal and establish whether the correction decreases variation in your experimental setup.

RELATED PRODUCTS

Item	Catalog No.
LightSwitch Assay Reagent™ (1000 assays)	LS100
GoClones™ Promoters	S7- - - - -
GoClones™ Promoter Controls	S79- - - -
GoClones™ 3'UTRs	S8- - - - -
GoClones™ 3'UTR Controls	S89- - - -
LightSwitch Transfection Optimization Kit	TFXOPT
FuGENE HD® Transfection Reagent	F500

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