Split Glow
Cell Assay Reagent

(Code : PXR-SG001)

Handling Instructions

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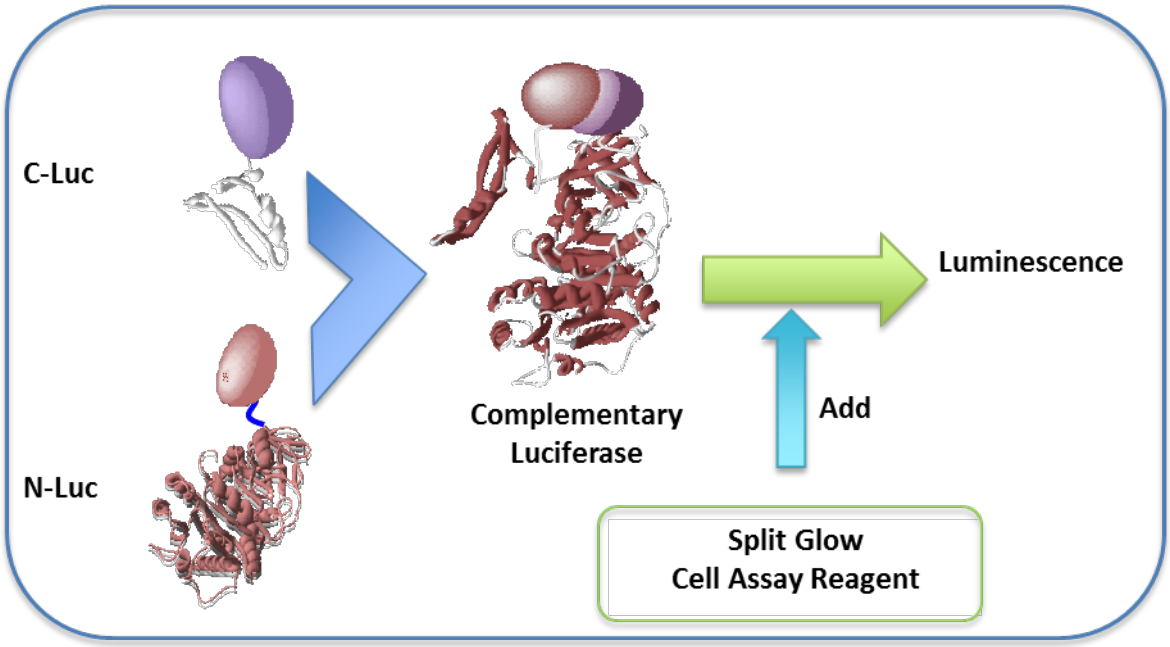
Note : This product is for research use only. Not intended for human or animal diagnostic or therapeutic uses.

Introduction

The split luciferase complementation assay is based on the principle that N- and C-terminal domains of luciferase alone do not emit luminescence, however, if fragments of the fused reporter protein are brought within proximity, they spontaneously refold to generate a detectable signal. The Split Glow Luciferase Reagent (Catalog No. PXR-SG001) is optimized to sensitively detect cell-based click beetle split luciferase activity over a prolonged period of time.



Kit Contents

1. Split Glow Cell Assay Reagent	10 mL
2. Handling Instructions	1 copy

Additional Reagents & Material

- Cell Culture Flask
  75cm² TC-Treated Culture Flask (Corning, 430641) or equivalent.
  Please note that untreated culture vessels do not support proper adherence of HEK293 cells. The use of untreated vessels may adversely impact results.

- Culture Media
  D-MEM with 4.5 g/L glucose, L-glutamine, and sodium bicarbonate, without sodium pyruvate, liquid, sterile-filtered, suitable for cell culture and sodium (Sigma, D5796), (Wako, 044-29765) or equivalent.

- Fetal Bovine Serum
  FBS (10437-028, Life Technologies) or equivalent.

- Antibiotics
  10,000 units penicillin and 10 mg streptomycin/mL, sterile-filtered (Sigma, P4333) or equivalent.

- Trypsin-EDTA (diluted to 1X final concentration).
  0.5% Trypsin-EDTA (10X), (15400-054, Life Technologies) or equivalent.

- Phosphate buffered Saline (without Ca^{2+}, Mg^{2+})
  PBS, pH 7.2 (Life Technologies, 20012-027) or equivalent.

- Media for Ligand stimulation and for Split Glow Cell Assay Reagent Dilution
  D-MEM with 4.5 mg/L glucose and sodium bicarbonate, without L-glutamine, sodium pyruvate, and phenol red (Sigma, D1145), (Wako, 040-30095) or equivalent.

- Selective antibiotic agents
  - G418 (Merck Millipore, 345812)
  - Zeocin™ (Life Technologies, R250-05)

- Assay plate
  96 Well Flat Clear Bottom White Polystyrene TC-Treated Microplates (Corning, 3903) or equivalent.
  Please note that untreated culture vessels do not support proper adherence of HEK293 cells. The use of untreated vessels may adversely impact results – including a reduction in signal intensity.

- Luminescence Plate reader
  Tri Star LB941 (Berthold Technologies), EnVision (PerkinElmer), Synergy (BioTeK), or equivalent.

[Complete Culture Media]

D-MEM with 4.5 g/L glucose, L-glutamine, and sodium bicarbonate, without sodium pyruvate (Sigma, D5796) or equivalent containing 10 % (w/v) FBS supplemented with 100 units penicillin, 0.1 mg streptomycin/mL, 0.8 mg/mL G418 and 0.05 mg/mL Zeocin™.

[Handling Procedure for Frozen Cells]

To ensure high viability, thaw the vial and initiate cultures as soon as possible upon receipt. If prolonged storage is necessary, please store vials in the vapor phase of liquid nitrogen.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of water. Thawing should be rapid and should not exceed 2 minutes.

2. Remove the vial from the water bath as soon as the contents are partially thawed (the outside is thawed but ice still exists inside), and sterilize by spraying with 70% ethanol. All operations from this point forward should be carried out in a sterile environment.

3. Transfer the vial contents to a centrifuge tube containing 9 mL of pre-warmed (37°C) complete culture medium. Pellet the cells at 180 x g for 2 minutes at room temperature.

4. Resuspend the cell pellet in 15 mL of the complete medium and dispense into a surface treated 75 cm^2 flask. Incubate the flask in a humidified atmosphere containing 5% CO_2 and maintained at 37°C.

5. On the following day, confirm the cells are attached to the flask and exchange the medium.

[Subculturing]

1. Begin propagating the cells when the density approaches 80 to 90 % confluency.

2. Prewarm the media and Trypsin-EDTA solution to 37°C.

3. Remove and discard culture medium and rinse the cell layer with PBS.

4. Add 2 to 3 mL of Trypsin-EDTA solution to the flask and incubate at 37°C for 3 minutes. to disrupt cellular attachment. Using an inverted microscope, inspect the cells to determine if they remain attached to the vessel. If they remain attached, the flask may be gently tapped to encourage detachment.

5. Add 8 to 10 mL of complete growth medium and aspirate the cells by gently pipetting.

6. Pellet the cells at approx. 180 x g for 2 minutes at room temperature.

7. Aspirate the supernatant and resuspend the cells in 0.5 to 1 mL culture media.

8. Add appropriate aliquots of the cell suspension to new culture vessels.

9. Incubate cultures at 37°C until the density approaches 80 to 90 % confluency.

10. Repeat the above steps as necessary.

To obtain a reliable and stable signal, we recommend that cells be used following 2 to 3 serial passages. The cells should be expanded and stored at $5x10^6/500\mu L$ (storage temperature: -150°C in liquid nitrogen). We recommend to use each cell lines up to 20 passage.

- GPCR assay

1. Harvest the cells by treating with Trypsin-EDTA. Pellet the cells (180 x g for 2 minutes at room temperature) and wash twice with fresh complete medium.

2. Resuspend cells in complete medium and adjust the density to 2 x 10^5 cells/mL.

3. Add 100 μL/well cell suspension to a 96 well plate (2 x 10^4 cells per well) and incubate for 48 hours in a humidified CO_2 atmosphere maintained at 37°C.

4. Prepare the ligand in D-MEM without phenol red.
   * in situations where the stimulation period will exceed 4 hours, 1% FBS should be added.

5. Remove the culture media and replace with the ligand containing medium.

6. After the desired period of stimulation, remove the medium completely and freeze the plate at −80°C for at least 30 minutes.

7. Thaw the Split Glow Cell Assay Reagent (50μL/well will be required) at 4°C and dilute 2-fold with D-MEM lacking phenol red. Mix gently by inversion to avoid bubbling and warm to room temperature. The diluted reagent can be restored at -30°C.

8. Thaw the frozen plate at room temperature for 5 to 10 minutes and add diluted Split Glow Cell Assay Reagent (100μL/well).

9. Gently mix with a rotary shaker for 5 minutes and measure luminescence*.

*Please cover the bottom of the plate. Transparent bottom is not suitable for luminescence measurements.

- Hif-1 assay

1. Harvest the cells by treating with Trypsin-EDTA. Pellet the cells (180 x g for 2 minutes at room temperature) and wash twice with fresh complete medium.

2. Resuspend cells in complete medium and adjust the density to 4 x 10^5 cells/mL.

3. Add 50 μL/well cell suspension to a 96 well plate (2 x 10^4 cells per well).

4. Prepare the inhibitor in the complete medium.
   * in the case of Acriflavine, the recommending concentration is 10μM (5μM in final concentration).

5. Add 50 μL/well inhibitor solution or vehicle control to a 96 well plate and incubate for 24 hours in a humidified CO_2 atmosphere maintained at 37°C.

6. Remove the culture media and replace with the ligand containing medium.

7. After the desired period of stimulation, remove the medium completely and freeze the plate at −80°C for at least 30 minutes.

8. Thaw the Split Glow Cell Assay Reagent (50μL/well will be required) at 4°C and dilute 2-fold with D-MEM lacking phenol red. Mix gently by inversion to avoid bubbling and warm to room temperature. The diluted reagent can be restored at -30°C.

9. Thaw the frozen plate at room temperature for 5 to 10 minutes and add diluted Split Glow Cell Assay Reagent (100μL/well).

10. Gently mix with a rotary shaker for 5 minutes and measure luminescence*.

*Please cover the bottom of the plate. Transparent bottom is not suitable for luminescence measurements.

- IKK assay

1. Harvest the cells by treating with Trypsin-EDTA. Pellet the cells (180 x g for 2 minutes at room temperature) and wash with fresh complete medium.

2. Resuspend cells in complete medium and adjust the density to 6 x 10^5 cells/mL.

3. Add 100 μL/well cell suspension to a 96 well plate (6 x 10^4 cells per well) and incubate for 48 hours in a humidified CO_2 atmosphere maintained at 37°C.

4. Prepare the ligand (1.25μg/mL TNFα in D-PBS(-)).

5. Add 2μL of 1.25μg/mL TNFα to the culture media directly (Final conc. 25ng/mL).

6. After 1 hour in 37°C, 5 % CO_2, remove the medium completely and freeze the plate at −80°C for at least 30 minutes.

7. Thaw the Split Glow Cell Assay Reagent (50μL/well will be required) at 4°C and dilute 2-fold with D-MEM lacking phenol red. Mix gently by inversion to avoid bubbling and warm to room temperature. The diluted reagent can be restored at -30°C.

8. Add diluted Split Glow Cell Assay Reagent (100μL/well).

9. Gently mix with a rotary shaker for 5 minutes and measure luminescence*.

*Please cover the bottom of the plate befor adding Reagent. Transparent bottom is not suitable for luminescence measurements.

Storage Conditions

-80°C.

Others

Patent: JP4849698 (B2), US8470974 (B2)