Luciferase Assay in Drosophila SL2 Cells

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Reagents:

1. Plasmids:
2. Transfection reagents: X-tremeGENE HP DNA Transfection Reagent, Roche
3. Medium: HyQ CCM3, UTECHProducts
4. Antibiotic: gentamicin sulfate, Cellpro.
5. 96 well assay plate: Corning Inc.
6. Dual-Glo Luciferase Assay kit: Promega

Protocol:

Day 1 – Prepare cells

1. Pick up healthy SL2 cells and dilute appropriately for next day use.

Day 2 – Transfection

2. Seed SL2 cells into 6 well plate at around 80% confluences.
3. Allow plates to incubate for 2~3 hours at 24ºC.
4. Transfect cells using X-TremeGENE HP DNA Transfection Reagent.
    a. For each transfection reaction, mix plasmid DNA into 100ul of antibiotic-free HyQ CCM3 medium in 1.5ml Eppendorf tube.
    b. Directly pipet X-TremeGENE HP DNA Transfection Reagent into medium containing plasmid DNA with 2:1 ratio of ul transfection reagent to ug DNA.
    c. Incubate for 20 minutes at RT.
    d. After 20 minutes incubation, suck off medium of 6 well plate and rinse cells with antibiotic-free medium once. Add 1.5ml of fresh antibiotic-free medium into each well.
    e. Add transfection reaction into cells drop by drop with shaking plate gently.
    f. Incubate plate for 8 hours at 24ºC.
    g. After incubation, add 1.5ml of medium containing 25ug/ml of gentamycin. Continue to incubate cells at 24ºC for 48~72 hours.

Day 5 – Dual-Glo Luciferase Assay

5. Remove 6 well plate containing cells from incubator. Transfer 75ul cells into each well of 96 well assay plate.
6. Combine lyophilized Luciferase substrate with Luciferase buffer to make Luciferase Reagent. Mix by inversion until the substrate is thoroughly dissolved.
7. Add 75ul of Luciferase Reagent into each well and mix. Incubate 10 minutes at RT.
8. After incubation, measure firefly luminescence.
9. Dilute the Stop & Glo substrate 1:100 into an appropriate volume of Stop & Glo buffer to make Stop & Glo Reagent.
10. Add 75 ul of room temperature Stop & Glo Reagent to each well and incubate for 10 minutes at RT.

11. Measure the Renilla luminescence in the same order as the firefly luminescence was measured.

To optimize the results:

At step 4: treat cells gently to avoid losing cells.

At step 4-b: use 1:1~4:1 ratios of ul transfection reagent to ug DNA for different type of cells.

At step 6: Luciferase Reagent should be store at -70ºC.

At step 8: measure the luminescence within 2 hours of addition of Luciferase Reagent.

At step 9: make Stop & Glo Reagent immediately before use.

At step 10: Stop & Glo Reagent should be added into plate wells within 4 hours of addition of Luciferase Reagent. Renilla luminescence should be measured within 2 hours of addition of Stop & Glo Reagent.