Split-Gaussia Protein Complementation Assay in presence of a third protein under viral infection

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Abstract

This protocol aims to assess:

- the interaction between two proteins harboring two parts of the gaussia luciferase (Glu1 and Glu2)

- in presence of a third protein co-expressed

- in presence of a rabies virus

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Protocol

Day 1 - Plate cells

Step 1.

1. Plate HEK-293T cells in 96-well plates with 25 000 cells per well in 100 µL of DMEM+10%FBS.

2. Incubate for 24h at 37°C, 5% CO2.

Day 2 - infection (optional)

Step 2.

Infection of the cell with rabies virus 3 hours before the transfection.

1. Remove 50 µL of media

2. Add 50 µL of virus at MOI 1 in DMEM without FBS.

3. Incubate for 3h at 37°C, 5% CO2

Day 2 - transfection with Lipofectamine 2000

Step 3.

1. Prepare a 25 µL DNA mix with 100 ng for a Glu1-tagged protein, 100 ng for a Glu2-tagged protein, 100 ng for a cmyc-tagged protein and 2 ng for a plasmid expressing the firefly luciferase (ex: pGL4.50) per well in DMEM without FBS. at least 3 wells per condition.

2. Add 0.5 µL of Lipofectamine 2000 to 25 µL of DMEM witout FBS.

3. Add the Lipofectamine to the DNA mix and incubate for 30 min.

4. Add 50 µL gently to the cells.

5. Incubate at 37°C, 5%CO2 for 48h

Day 4 - PCA

Step 4.

1. Remove all the medium

2. Add 50 µL of Renilla Luciferase Assay Lysis Buffer (Promega)

3. Incubate for 15 min at room temperature.

4. Add 15 µL of cell lysate to 30 µL of Renilla Luciferase Assay Reagent, read immediately.

5. Add 15 µL of cell lysate to 15 µL of Firefly Luciferase Assay Reagent, read immediately.

Analysis

Step 5.

1. Normalize each Gaussia luciferase value according to the Firefly luciferase value.

2. Determine the Normalized Luminescence Ratio (NLR) as follow:

NLR = signal (Glu1-A + Glu2-B) / [signal (Glu1-A + Glu2) + signal (Glu1 + Glu2-B)]

3. Perform a logarithmic transformation of the NLR values.