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Luciferase Assay pH-dependent Fox-activity

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Protocol status: Working
We use this protocol and it's working

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Abstract

Working protocol for how to perform luciferase assay. Worked for Fox reporter

Materials

- Generic tissue culture materials
- Cell line of choice
- Luciferase plasmid and Renilla control plasmid
- Dual-Glo luciferase kit (promega)

Safety warnings

No safety warnings

Before start

Read entire protocol before starting

Plate Cells

1 1. Plate 500K cells/well for each conditon

2 Incubate o/n before transfection

Transfection

3 For each transfection condition create the following tube mixtures. (**Volumes are for 1 transfection per condition and can be scaled up)**
a. Tube 1: 125uL Optimem + 4uL lipofectamine 3000
b. Tube 2: 125uL Optimem + 2uL p3000 reagent + 1 ug total DNA

4 flick and incubate 5 minutes

5 Mix together tube 1 and tube 2 and incubate 15 min

6 Add dropwise to cells and incubate o/n

7 Incubate 8 hours

8 Change media to fresh media or media + 10mM EIPA

48 hours incubation

9 Incubate cells for 48 hours after transfection. Change media at 24 hours post transfection with fresh media and fresh media + EIPA

Luciferase Assay

10 Slowly thaw luciferase reagent in room temp water

11 Wash gently each well with PBS

12 Add 500uL of luciferase reagent directly to plate and nutate for 10 min RT

13 Scrape each well with cell scraper and put into fresh microfuge tube

14 Spin down 13,000 rpm 5 min at RT

15 Add 100uL of supernatant to 4wells of white 96well plate. Wait 10 minutes

16 Read luciferase on plate reader

17 For Renilla reading, calculate reagent needed for 100uL each well using a 1:100 dilution of reagent: buffer

18 Add 100uL of renilla reagent to each well and wait 10 min before reading