Luciferase assays in OT-1 hybridoma cells

June Round
UCLA

Method Article

Keywords: luciferase

Posted Date: January 29th, 2007

DOI: https://doi.org/10.1038/nprot.2007.68

Abstract

Introduction

This protocol was used in the above _Nature Immunology_ paper.

Reagents

Firefly Luciferase reporter vector \(we have NFAT \(K. Siminovitch, Samuel Lunenfeld Research Institute of Medicine), NFkappaB \(G. Cheng, UCLA) and AP1 \(Panomics, LR0002) Renilla vector \(PRLSV40, Promega) Electroporation cuvettes \(Bio-RAD; 0.4cm ;165-2088) Complete media Dual Luciferase kit \ (Promega; cat no E1910)

Procedure

1) For every transfection use half of a large \(175 cm) confluent flask \(2 transfections/ flask) 2) Collect cells and resuspend in fresh complete media at a volume of 0.5 ml / transfection \(example 4 transfections, need 2 confluent large flasks, resuspend in a volume of 2 ml of complete media) 3) Place 0.5 ml of cells in one cuvette and place on ice 4) For Dlgh1- I usually transfect at least two concentrations \(from 1µg-5µg) Luciferase reporter vector = 200 ng Renilla vector= 0.5-1µg 5) Mix DNA with cells, ensure that you flick the cuvette a couple of times to mix 6) Let sit on ice at least 5 minutes – do not shorten this time- can go up to fifteen minutes 7) Electroporate at 240 volts at 960 mF and place 0.5ml of cells immediately into 10 ml of fresh complete media in a small flask. Allow cells to sit in small flask overnight 8) Plate 5µg of anti-CD3 and 20µg of anti-CD28/ ml of PBS and aliquot 500µl into each well of a 6 well plate \(allow antibody to sit overnight). 9) Twenty-four hours post-transfection, place cells in 10 mL of fresh media and take half of the cells \(5mL) and put them in the antibody coated 6 well dish to be stimulated overnight. The other half should remain in the flask and will be the unstimulated sample. 10) Next day collect cells and spin down, resuspend in 200 µl of Passive Lysis Buffer \(Promega) and let sit at room temperature for twenty minutes. Freeze thaw the cells at least once to ensure complete lysis. 11) Spin down debris and take 50 µl from each sample of supernantant and place in a 5mL polystyrene round bottom tube \(BD 352008). 12) Using Program 30 on the luminometer \(Cheng lab, UCLA) read luciferase numbers by adding 50 µl of LAR \(Luciferase Assay Buffer, contained within Promega kit) immediately add 50 µl of STOP \(Stop and glow buffer, Promega) and read in the luminometer again to get Renilla luciferase numbers to use for normalization. 13) The first number for each sample is the experimental luciferase number while the second number from each sample should be used as a “loading control” ensuring equal amounts of DNA were added. 14) The luciferase number should be divided by the renilla number. This will usually give you a number less than 0, so multiply this number by 10000 \(make sure that you multiply each sample by the same number). These are the relative normalized numbers. You can either graph these numbers or calculate fold differences.