Developing a Luciferase-Based NF-κB Reporter Assay with Transiently Transfected HEK 293 Cells

Introduction

Firefly luciferase is widely used as a reporter molecule in cell based assays because of its sensitivity, dynamic range and ease of detection. Luciferase based assays have been developed for many different applications, and they can be incorporated into multiple assay formats.

One of the challenges to developing a luciferase assay for high throughput drug screening involves the need to generate large numbers of cells that contain the reporter DNA. The most common approach to cell based assay development involves creating stable cell lines, a costly, time consuming and labor intensive process that requires multiple rounds of selection and clonal isolation. In addition, if an assay requires coexpression of reporter and target molecules, cells need to undergo additional rounds of screening and exposure to multiple, harsh selection agents.

As an alternative to stable cell line development, the MaxCyte STX system provides a rapid, scalable method for introducing reporter and target molecules into cell lines and primary cells. Up to $1x10^{10}$ cells can be transfected in less than thirty minutes using MaxCyte's flow electroporation technology. Assay optimization is a straight forward process of performing several small scale electroporations with varying concentrations of plasmid DNA. After identifying a DNA concentration that yields optimal assay results at small scale, the electroporation process can be scaled up without impacting transfection efficiency or cell viability. Another advantage to the MaxCyte STX system is that multiple agents can be introduced into the cell at one time. Thus, cells can be co-transfected with plasmids encoding reporters, targets and accessory proteins. In addition to plasmid DNA, the MaxCyte STX provides an efficient method to load cells with siRNA, mRNA, proteins and other molecules. Cells transfected in bulk with the MaxCyte STX can be plated for immediate use or aliquoted and cryopreserved for future assay applications.

Here we show how the MaxCyte STX system can be used for rapid development of a luciferase based reporter assay in HEK 293 cells. We transfected cells with a reporter plasmid containing multiple NF-κB response elements linked to a minimal promoter controlling transcription of the firefly luciferase cDNA. The NF-κB transcription factor plays important roles in inflammation, immune responses, cell growth and apoptosis. Tumor necrosis factor alpha (TNFα), a key signal for inducing apoptosis in many cell types, is a potent activator of NF-κB responsive genes. In this study, we demonstrate that transiently tranfected cells exhibit robust, consistent and dose-dependent luciferase expression in response to TNFα stimulation.

Materials

HEK 293H cells (Invitrogen11631-017)
DMEM (Lonza 12-614F)
Fetal Bovine Serum (FBS) (Lonza14-501F)
L-glutamine (Lonza 17-605E)
Non-essential amino acids (NEAA)(Lonza 13114E)
Penicillin-streptomycin (pen/strep) (Lonza 17602E)
Phosphate buffered saline (PBS)(Lonza 17-516F)
Trypsin (Invitrogen 25300-054)
Tissue culture flasks (Corning 430825)
96 well plates (Corning 3610)
Cignal™ NFκB Reporter Kit (SABiosciences CCS-013L)
Tumor Necrosis Factor alpha (TNFα) (Peprotech, cat # 14832962)
Dual-Glo® Luciferase Assay System (Promega E2920)

Methods

Cell culture:
HEK 293H cells were plated in T150 flasks and cultured at 37°C with 5% CO_2 in DMEM supplemented with 10% FBS, 1% pen/strep, 1% NEAA, and 1% L-glutamine. Cells were passaged every 2-3 days and maintained at 50-90% confluency. The cells were split one day prior to electroporation to ensure that they were healthy and in log phase growth at the time of transfection.

Electroporation:
Cells were harvested for electroporation using 0.05% trypsin. After neutralizing the trypsin with an equal volume of complete medium, cells were pelleted at 250 X g for 10 min., rinsed in MaxCyte electroporation buffer (2-5X final electroporation volume), pelleted again, and then suspended in MaxCyte buffer at a density of $5x10^7$ cells/mL. Cells were then mixed with plasmid DNA (dissolved in sterile, distilled water) and transferred to OC-100 processing assemblies (PAs). After electroporation using the preset "HEK 293" protocol, transfected cells were transferred from PAs into sterile 1.5 mL tubes and placed in a 37°C water bath for 20 min. Following the 20 min. incubation, cells were diluted into complete culture medium and transferred to 96 well plates.

Luciferase Assays:
Six hours after plating, cells were treated with TNFα dissolved in dH_2O. Control cells were treated with dH_2O alone. Eighteen hrs later, luciferase activity was measured on a FLUOstar OPTIMA plate reader (BMG Labtech).

Results

Figure 1 shows firefly luciferase activity levels in HEK 293 cells that were transiently transfected with a reporter plasmid containing NF-κB response elements then treated with a single concentration of TNFα. These data illustrate how MaxCyte STX users can control assay sensitivity at multiple levels. The dynamic range of the assay can be modulated by varying the concentration of loading agent, and because the MaxCyte STX system is not restricted to a particular multiwell format, varying numbers of transfected cells can be plated in standard or specialized plates that are used with a wide range of detection systems.

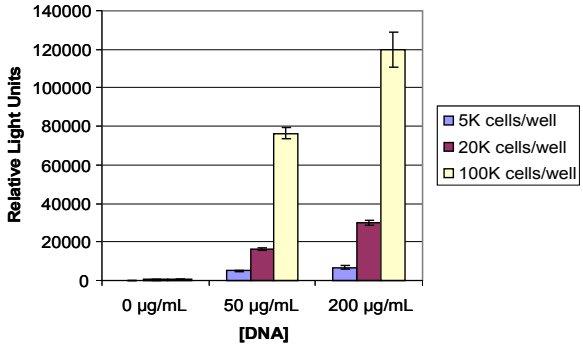

Figure 1. Controlling assay sensitivity via loading agent concentration and cell plating density. HEK 293 cells concentrated in MaxCyte's electroporation buffer were mixed with 50 µg/mL or 200 µg/mL of plasmid DNA encoding an NF-κB responsive luciferase reporter plasmid and transfected by static electroporation. Control cells were electroporated in the absence of DNA. Cells were plated in 96 well plates with opaque walls at 5,000, 20,000 or 100,000 cells/well and then assayed for luciferase expression after overnight exposure to 10 ng/mL of TNFα. Error bars indicate standard deviations in three replicate wells.

In a separate experiment, HEK 293 cells were transfected with two different concentrations of a mixture of the NF-κB reporter plasmid combined with a plasmid expressing renilla luciferase from a constitutive promoter. Renilla luciferase is commonly used as a control for normalizing transfection efficiencies in luciferase-based reporter assays. Six hours after electroporation, the transfected cells were exposed to increasing concentrations of TNFα, and luciferase activity was measured the following day.

Figure 2A shows that both sets of transfected cells exhibited concentration-dependent expression of firefly luciferase in response to treatment with increasing amounts of TNFα. As in the previous experiment, the sensitivity of the assay was increased by raising the loading agent concentration. However, when firefly luciferase activity was normalized using renilla luciferase, the two sets of transfected cells showed nearly identical responses to TNFα stimulation, These results illustrate reproducibility and consistency of the MaxCyte STX transfection process (Figure 2B).

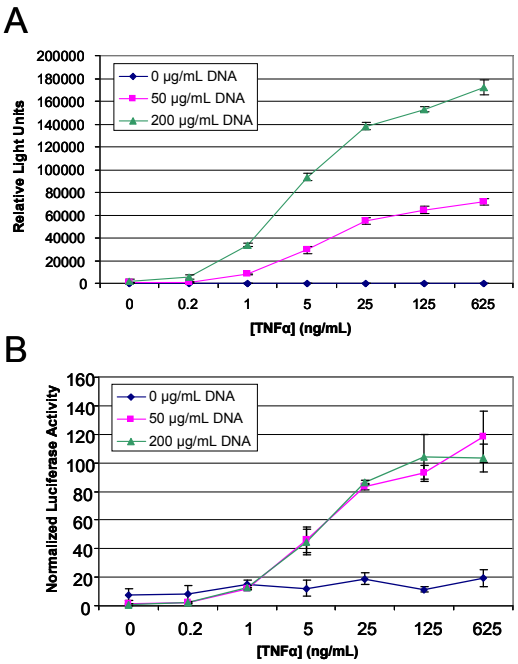

Figure 2. Reproducible, concentration-dependent response to TNFα stimulation in transiently transfected cells. HEK 293 cells were transfected with 50 µg/mL or

200 µg/mL of two plasmids, an NF-κB -responsive firefly luciferase plasmid and a constitutively active renilla luciferase plasmid, combined in a 40:1 weight to weight ratio. Transfected cells were plated in 96 well plates (10,000 cells per well). After overnight exposure to TNFα, firefly and renilla luciferase levels were measured sequentially in each well. A. Firefly luciferase activity. B. Firefly luciferase activity normalized to renilla luciferase activity. Error bars indicate standard deviations in three or two replicate wells of transfected or non-transfected cells, respectively.

Summary

- ✓ The MaxCyte STX system offers an efficient, robust and scalable method for introducing reporter and target molecules into cell lines and primary cells.
- ✓ Cells transfected with the MaxCyte STX system can be used in a variety of assay applications and with multiple detection systems. There are no restrictions on the plating format or on the number of cells that are used per assay.
- ✓ MaxCyte STX users have tight control over assay performance based on the concentration of loading agent that is added to the electroporation reaction.
- ✓ Through the application of invariant, tightly controlled electrical parameters, the MaxCyte STX electroporation technology allows users to achieve reproducible levels of transfection efficiency and assay performance.
- ✓ Rapid introduction of plasmids into cells enables MaxCyte STX users to generate assay results within 24 hrs of transfection.