Chemiluminescence imaging of cells (CLIC)

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Method Article

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Abstract

Here we describe a simple, sensitive and robust chemiluminescence-based immunoassay (chemiluminescence imaging of cells; CLIC) for relative quantification of proteins in cells. We first employed this method to quantify complement activation in cultured mammalian cells, and to quantify membrane protein expression, shedding, binding and internalization. Moreover, through specific membrane permeabilization we were able to quantify both cytosolic and nuclear proteins, and their translocation. We validated the CLIC quantification method by performing parallel experiments with other quantification methods like ELISA, qPCR, and immunofluorescence microscopy. The workflow of the immunoassay was found to be advantageous in certain instances when compared to these quantification methods. Since the reagents used for CLIC are common to other immunoassays with no need for specialized equipment, and due to the good linearity, dynamic range and signal stability inherent to chemiluminescence, we suggest that this assay is suitable for both small scale and high throughput relative protein quantification studies in whole cells

Introduction

In this protocol we describe a simple and robust relative quantification method of proteins in cultured cells through chemiluminescence imaging of cells (CLIC). CLIC can be used for relative quantification of complement activation on human cells and bacterial cells, the expression level of membrane receptor proteins, and the shedding of membrane proteins. CLIC can be used to rapidly investigate the efficacy of RNA knockdown at the protein level. Furthermore, it can be used to quantify the relative levels of cytosolic, nuclear, and membrane proteins and quantify differences in protein localization between cytosolic and nuclear compartments.

In certain instances, using CLIC was advantageous over commonly used immunoassays. Assays that use fluorescence detection methods often suffer from a high background signal due to cell or reagent autofluorescence that makes quantification of subtle changes difficult in adherent cells.

The minimal manipulation of cells in CLIC helped to yield faster and more reproducible results compared to our previous experience with Western blots and dot blots, likely because CLIC does not use several steps that can adversely affect reproducibility

Reagents

- Cell Culture plates.

- Paraformaldehyde (PFA).

- Tris buffered saline (TBS)

- Blocking solution (TBS containing 5% [w/v] bovine serum albumin [BSA], 5% serum from the same species as the secondary antibody.

- Primary Antibodies against target protein.

- Secondary Antibodies against species of the primary antibodies, conjugated to Horse radish peroxidase (HRP).

- Enhanced chemiluminescence substrate (ECL).

Equipment

- Cell culture incubator.

- ChemiDoc MP imaging system (Bio-rad), or any imager with cooled CCD cameras (commonly used to develop Western blots)

- Shaker for washing plates.

- Image analysis software such as ImageJ.

Procedure

- Cells are cultured in 6, 12, 24, or 96-well transparent cell culture plates.

- Cells are fixed with freshly made 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) for 30-60 mins at room temperature.

- Cells are washed twice in Tris buffered saline (TBS).

- Cells are incubated with a blocking solution (TBS containing 5% [w/v] bovine serum albumin [BSA], 5% serum from the same species as the secondary antibody, and the appropriate permeabilizing agent depending on the expected subcellular localization of the protein, as detailed below) for 30-60 mins at room temperature.

- Cells are incubated with primary antibodies in blocking solution overnight at 4°C.

- The next day cells are washed 3-5 times, 3 mins each in TBS on a shaker.

- Cells are incubated with HRP-conjugated secondary antibodies in blocking solution for 2-4 hours at room temperature.

- Cells are washed 3-5 times, 3 mins each in TBS on a shaker.

- Cells are incubated with enhanced chemiluminescence (ECL) substrate (SuperSignal West Pico Chemiluminescent Substrate; Pierce) for 2-5 mins.

- Cells are placed in ChemiDoc MP imaging system (Bio-rad), and images are taken using the Chemiluminescence application *Chemi Hi Sensitivity* and the *auto exposure* option.

- After imaging, the cells are washed twice in TBS to remove the ECL substrate and kept in TBS at 4°C for future stripping and re-probing if necessary.

- The image files aret analyzed using Fiji/ImageJ, see figure 1.

- In Fiji, first an area of interest (AOI) is chosen. The AOI used is typically a circular selection about 60% of the size of the well in the image, placed approximately in the center of each well to avoid the well edges as they are known to cause noise and variability in cell-based assays.

- The signal in each well is quantified using the *Measure* command under the *Analyze* tab. The AOI selection is then dragged to the next well to ensure that the size remained constant, and this process is repeated for each well. The AOI can be saved for future experiments using the “ROI manager” tool in ImageJ. The *Integrated density* values for each well is then recorded.

Troubleshooting

- We emphasize that the bottom of the well should be sufficiently covered with all solutions involved to avoid artefacts.

- The dilutions of the primary antibodies ranged from 1:200 to 1:2000 depending on the expected concentration of the protein of interest, while for the HRP-conjugated secondary antibodies a dilution of 1:2000 was used in most experiments.

Time Taken

- Time is calculated from the start of fixation of cells in the cell culture plates.

- Time taken varies depending on the antibody incubation times, which can be optimized for the target protein and antibodies used.

- Approximate shortest time 6 hours

- Approximate longest time 24 hours

Anticipated Results

CLIC is sensitive in detecting subtle differences in protein expression levels, protein binding or shedding to/from the cell membrane, and protein translocation to the nucleus. Chemiluminescence has no

substantial background signal in biological samples like that found with fluorescence, hence technical controls (for example those lacking a secondary antibody) must not show any significant background signal.

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Figures

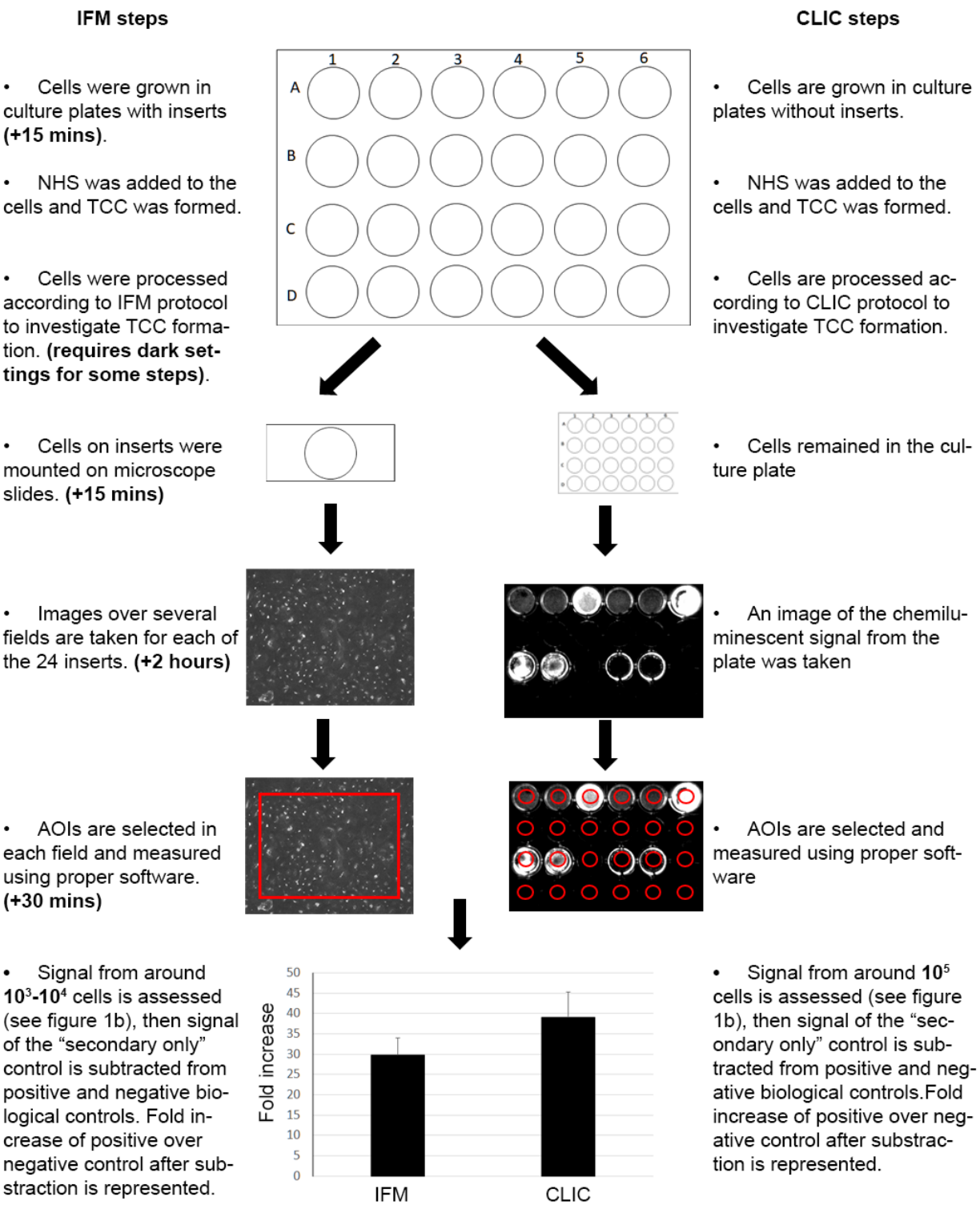

Figure 1

Comparing IFM and CLIC workflow in quantification of complement activation. IFM, immunofluorescence microscopy. CLIC, chemiluminescent imaging of cells. NHS, normal human serum. TCC, terminal complement complex. AOI, area of interest. Times indicated in the IFM steps are the approximate time increase of each step relative to CLIC.