Fluoro IndoBlu™

A cellular proliferation and viability assay

PROTOCOL

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I. Introduction:

Measurement of cell proliferation and viability is frequently used in clinical and experimental immunology as means of assessing cell activation in response to diseases, infections and environmental stimulations. Fluoro IndoBlu™ assay can be used for the measurement of cell proliferation in response to antigens, cytokines, growth factors, and mitogens. It can also be used for the analysis of cytotoxic effects of anticancer drugs, drug resistance, cytotoxic pharmaceutical compounds, and other toxic agents.

II. Assay Principle:

Cell Technology introduces a Fluoro IndoBlu™ reagent for cell proliferation and viability assay. Fluoro IndoBlu™ is an oxidation/reduction- based reagent that functions as a cell viability indicator by quantitatively measuring the reducing power of living cells. Fluoro IndoBlu™ is a cell-permeable reagent that is blue in color, but non-fluorescent. When added to cells, the IndoBlu™ reagent is modified by the reducing environment of the viable cell, it turns red in color and becomes highly fluorescent. This change can be detected using fluorescence or absorbance measurements. Fluoro IndoBlu™ is detected at Excitation = 530 nm and Emission = 590 nm. Absorbance is measured at 570nm.

Compared to the traditional cell proliferation assay, Cell Technology's novel in-cell-culture method is simple, fast and sensitive. The entire assay can be performed in a 96-well microtiter plate in 30 min and the best results are achieved in 3 hours.

III. Catalog # INDBLU 100-2:

A. Warnings and Precautions:

1. This kit is only for usage on cell lines or primary cell cultures.

2. For research use only. Not for use in diagnostic procedures.

3. Gloves, protective clothing and eyewear should be worn and safe laboratory practices followed.

B. Storage and Shelf Life:

1. Short term: Store the kit at 2-8°C for (1 –2 weeks), keep it away from light.
2. Long term: Store the kit at -20°C.

If stored and handled properly (see table below) the performance of this product is guaranteed until the expiration date stated on the kit.

C. Kit contents:

1. Part # 4024 IndoBlu™ Reagent : 10mL.
2. Optional: 96-well clear-bottomed black plate for fluorescent read-out

D. Additional Materials Required, But Not Supplied:

1. Solutions
   a. Di Water
   b. Phosphate Buffered Saline (PBS)
   c. Cell Culture Medium

2. Equipment and supplies
3. Fluorescent microscope
4. Fluorescent plate reader with Ex/Em= 530-570/590-600 nm/absorbance at 570 nm.
5. Centrifuge
6. Falcon tubes and 96-well microtiter plates. For fluorescent measurement use black opaque plates, for absorbance use clear tissue culture plates.

IV. Reagent Preparation:

1. Prepare sterilized 1X PBS
2. Prepare the appropriate cell culture medium according to your experimental protocol

V. Assay Protocol for Cell Proliferation:

The following is a suggested protocol and can be modified to suit your particular research needs.

1. Prepare Cells for Proliferation

1. Prepare cells according to your protocol and wash with 1X PBS twice.
   Cells should be cultured to a density not to exceed 1x 10^6cells/mL.

2. Plate cells in a 96- well plate at a concentration of 10,000 to 500,000 cells per well in a volume of 100µL cell culture medium.

   Note: Each cell line should be evaluated on an individual basis to determine optimal density for cell culture.

3. Prepare test reagent (mitogen, antigen, cytokine, growth factor etc.) at desired dose in cell culture medium. Add 100µl to the cells cultured in the 96-well plate.

   Include the following appropriate negative controls to measure background fluorescence:

   a. Media alone.
   b. Non-activated cells.

4. Incubate the cells along with the test reagent at 37°C in a CO_2 incubator for the appropriate length of time.

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2. Staining cells for proliferation

At the end of incubation, add 1/10 volume of Fluoro IndoBlu™, incubate the cells for 3 hours at 37°C. Take fluorescent readings between 30 min and 3 hours at Ex: 530-570/ Em: 590-600nm. Absorbance can be detected at 570nm within the same time frame.

3. Fluorescent plate reader

Analyze samples with Ex/Em 530/590 nm. Please note that if your plate reader is a bottom reader you will need to use black clear bottom plates.

VI. Data Examples:

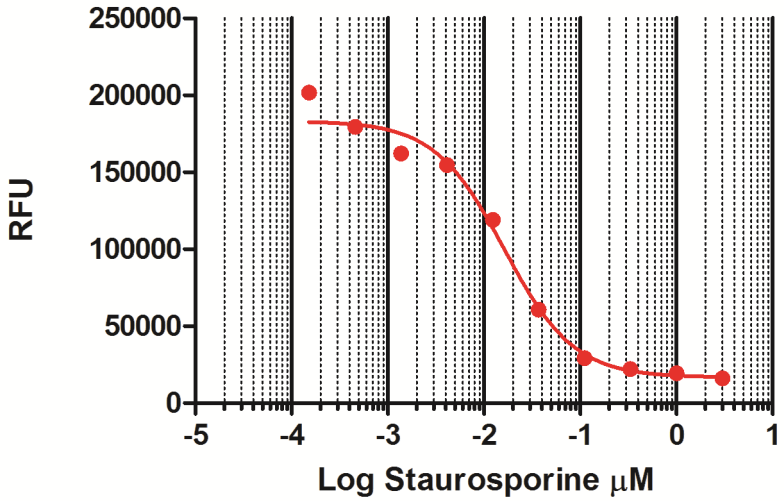

Figure 1: Staurosporine induced Jurkat cell viability assay. Titrated doses of staurosporine were added into seeded Jurkat (10,000 cells/well) cells in 96 well black opaque tissue culture plates and incubated at 37°C, 10% CO_2 for 24h. Fluoro IndoBlu™ (1/10 volume) was added into the cultured cells and incubated for 3h and fluorescence was detected at Ex: 530nm and Em: 590nm.
incubated at 37°C, 10% CO_2 for 24h. Fluoro IndoBlu™ (1/10 volume) was added in the cultured cells and incubated for 3h.

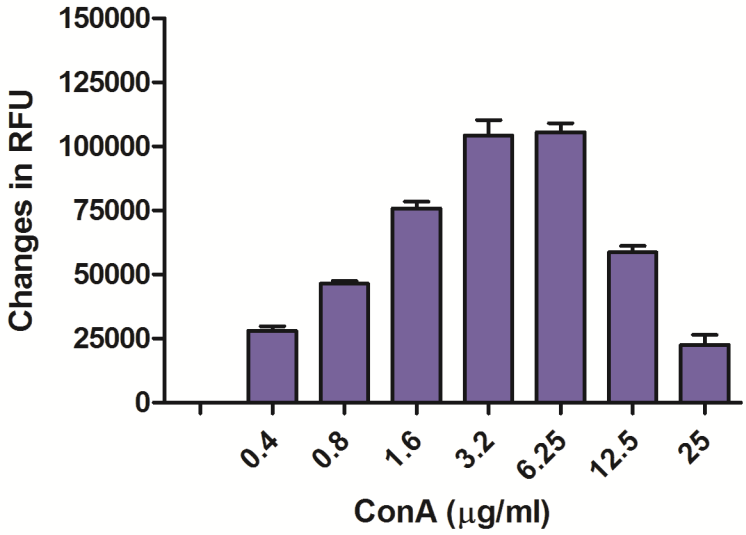

Figure 2: PBMC proliferation in response to mitogen Concanavalin A (Con-A) stimulation measured by Fluoro IndoBlu™. PBMC at 15,000 cells were cultured for 3 days in the presence of titrated amounts of Con A and tested for proliferation using the Fluoro IndoBlu™ assay. Fluoro IndoBlu™ (1/10 volume) was added to the cultured cells and incubated @ 37°C for 2h. Fluorescence was detected at Ex: 530nm and Em: 590nm.

VII. References

1. Al-Nasiry, Geusens N, Hassens M, Luyten C, Pijnengorg R (2007) The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. Hum Reprod 22: 1304.

2. Coban AY (2012) Rapid determination of methicillin resistance among *Staphylococcus aureus* clinical isolates by colorimetric methods. J Clin Microbiol. 2012 Apr 18. [Epub ahead of print].

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