FOR RESEARCH USE ONLY!

Cell Proliferation Assay Kit (Fluorometric)

1/15

(Catalog # K307-1000; 1000 assays; Store at -20°C)

I. Introduction:

BioVision's Cell Proliferation Assay Kit (Fluorometric) provides an easy and quantitative method to measure cell proliferation and cytotoxicity. The assay is based on a nuclear dye that specifically binds to nucleic acid in the cell and generates green fluorescence. The generated fluorescent intensity is directly proportional to the cell number, which can be quantified by measuring fluorescence (Ex/Em = 480/538 nm). This assay kit provides a just add-and-read, non-radioactive, and high-throughput method for cell proliferation and apoptosis. The assay is rapid and more sensitive than MTT, XTT, or MTS-based assays and can detect a wide linear range of 25-60,000 cells.

II. Application:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents, and other pharmaceuticals
- Assessment of physiological mediators and antibodies that inhibit cell growth

III. Sample Type:

Adherent or suspension cells

IV. Kit Contents:

Components	K307-1000	Cap Code	Part Number
Cell Lysis Buffer (5X)	25 ml	NM	K307-1000-1
Nuclear Dye (200X)	0.65 ml	Blue	K307-1000-2

V. User Supplied Reagents and Equipment:

- 96-well white tissue culture plate with clear bottom
- Multichannel or single channel Pipettes
- Multi-well spectrophotometer (Fluorescence reader)

VI. Reagents Preparation and Storage:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Cell Lysis Buffer (5X):** Store at 4°C or -20°C. Bring to room temperature (RT) before use.
- **Nuclear Dye (200X)**: For long-term storage, aliquot, and store at -20°C. Avoid freeze/thaw.

VII. Cell Proliferation Assay Protocol:

1. **Sample Preparation:** Culture cells (0.1 – 5 x 10^4 cells/well) in a 96-well white tissue culture plate in a final volume of 100 µl/well medium in the absence or presence of testing compounds. Incubate cells for the desired time period.

   Notes:

   a. For adherent cells, seed the cells one day before treating with testing compounds.

   b. Incubation time depends on the testing compounds.

   c. For toxicity assay, use more cells (e.g. 5 – 8x10^4 cells/well).

   d. (Optional) For low cell number and/or higher sensitivity (see Figure B), after incubation period, centrifuge cells at 400 x g for 5 min. and carefully remove supernatant using multichannel pipette (Don't use vacuum aspirator). Dilute Nuclear Dye (200X) with Cell Lysis Buffer (5X) and dH_2O to make 1X Nuclear Dye/Cell Lysis Buffer solution. Add 100 µl of 1X Nuclear Dye/Cell Lysis Buffer solution into each well. Gently shake the plate for 15 min. on a shaker at room temperature protected from light and directly proceed to step 3.

2. Prepare 25 µl/well of 5X Nuclear Dye/Cell Lysis Buffer solution by adding 0.625 µl of Nuclear Dye (200X) to 25 µl of Cell Lysis Buffer (5X). Make as much as needed. Mix. After incubation, directly add into each well. Gently shake the plate for 15 min. on a shaker at room temperature protected from light.

3. Measure fluorescence of treated and untreated cells using a microtiter plate reader at Ex/Em = 480/538 nm.

   Notes:

   a. Assay has a linear range up to 60,000 cells. Depending on the cell type, adjust the cell number to make sure that fluorescence reading is within the linear range.

   b. Serial dilutions of testing cells can be used to make standard curve.

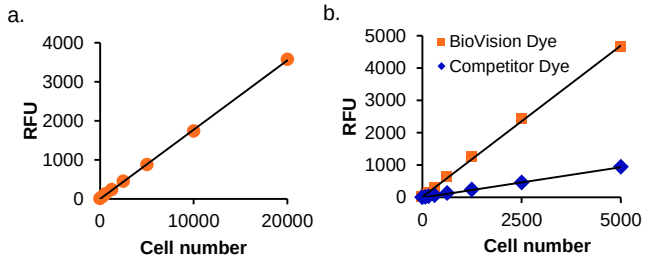

Figure: Cell proliferation assay. a) 5X Nuclear Dye/Cell Lysis Buffer solution was directly added to the serially diluted Jurkat cells in culture medium. Cells were incubated for 15 min. b) For low cell number and/or high sensitivity, cells were spun down to remove the medium before adding 1X Nuclear Dye/Cell Lysis Buffer solution or competitor dye. Cells were incubated for 15 min. Assays were performed according to the kit protocol.

VIII. RELATED PRODUCTS:

Quick Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
MTS Cell Proliferation Colorimetric Assay Kit (K300)
Ready-to-use Cell Proliferation Reagent, WST-1 (K304)
EZCell™ Cell Cycle Analysis Kit (K920)
Annexin V Apoptosis Kits (K101-K104)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
EZViable™ Calcein AM Cell Viability Assay Kit (Fluorometric) (K305)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ATP Colorimetric/Fluorometric Assay Kit II (K354)
Quick Cell Proliferation Colorimetric Assay Kit Plus (K302)
EZCell™ Cell Migration/Chemotaxis Assay Kits (K906-K912)
Live-Dead Cell Staining Kit (K501)
VisionBlue™ Quick Cell Viablility Fluorometric Assay Kit (K303)
Senescence Detection Kit (K320)
EZSolution™ 7-Aminoactinomycin D (2727)
StayBrite™ Highly Stable ATP Bioluminescence Assay Kit(K791)
ADP Colorimetric Assay Kit II (K356)
ADP Colorimetric/Fluorometric Assay Kit II (K355)

FOR RESEARCH USE ONLY! Not to be used on humans.