FT-669412

UptiBlue™ viable cell counting reagent

Features

UptiBlue reagent is a safe, non-toxic aqueous dye that designed to assess cell viability and cell proliferation of various human and animal cell lines, bacteria and fungi. The bioassay can also be used to establish relative cytotoxicity of agents within various chemical classes.
UptiBlue assay is as sensitive as [3H] thymidine assay [r,r] Depending on the cell types, UptiBlue can detect as few as 40 cells with reproducible and sensitive signal.

UptiBlue viable cell counting reagent UP669412, 25 ml
UP669413, 100 ml

Storage:(L) +4°C
Stable 12 months at room temperature, 20 months at 2-8°C, or indefinitely at -70°C.
Protect from light. Storage of UptiBlue at room temperature under lighted conditions adversely affects its absorbance properties.
When stored frozen, warm UptiBlue reagent to +37°C upon thawing and mixed well to be assured of complete resolubilization.

The UptiBlue Assay offers many advantages over conventional cell (MTT, XTT…) and radioactively-labeled incorporation assays :

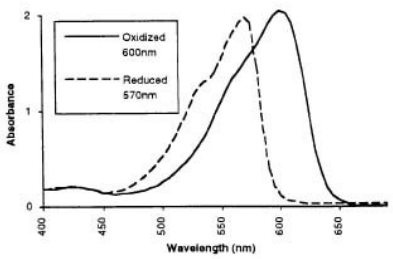
Features	**Benefits**
Fluorescent/Colorimetric Fluorescence is monitored at **530-560nm** excitation wavelenght and **590 nm** emission wavelenght	.Allows **choice of detection method**: either with a spectrophotometer or a spectrofluorometer .**no interference** from the presence of 10% fetal bovine serum, nor from phenol red in the growth medium.
Water soluble	.**No extraction** required
Works on suspension or attached cell lines	.**No centrifugation** required
Fewer steps	.**Time saving**, easily adaptable to **automation:** use either a standard spectro or fluorophotometer, or a microplate reader
Stable	.Allows for continuous cell growth monitoring, **kinetic studies**, **incubation time of days**
Non-toxic to cells	.Less likely to interfere with normal metabolism
Non-toxic to technician	.Safe, disposable, less regulation

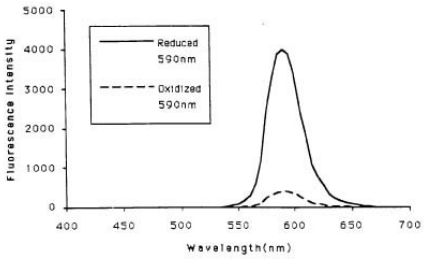
Quality Control

UptiBlue is supplied as a sterile indigo coloured liquid.

Control of UptiBlue reagent. Absorbances values.
In Uptima standard conditions*,
absorbances values are:
540nm 0.145 +-0.002
570nm 0.225+-0.003
600nm 0.313+-0.004
630nm 0.0016+-0.002

*0.4ml of UptiBlue reagent
with 10ml of phosphate buffer
0.1M pH7.4





It is difficult to propose a standard test for the reduced form, because it is very unstable in water. Absorbance / Fluorescence to be expected for a particular experiment can be determined as suggested: prepare 1X UptiBlue reagent in media. Reduce it by autoclaving for 15min. Allow to cool. Swirl and measure absorbance.
Rem: Fluorescences units are arbitrary and the scale used varies widely from one instrument to another.

Directions for Use

It is important to conduct a cell number titration assay (procedure 1) for each particular cell line of your interest to identify the optimal number of cells. See protocol for determining the cell plating density.

1-General Procedure for determining Length of Incubation Time and Plating density for a Cell Line

1.Harvest cells that are in log phase growth stage and determine cell count. Plate cells at desired density, and/or at various densities, some dilutions being above and below the cell density expected to be used.
I.e. for a microplate assay, plate cells in 100µL medium into 96-well tissue culture plates by conducting cell number titration in the range of 40 to 10 000 for adherent cells and 2 000 to 500 000 for suspension cells.
For background control, use 100µL medium without cells.

2.Aseptically add UptiBlue in an amount equal to 10% of the culture volume (in tubes or in microplate wells).

3.Return cultures to incubator. Measure fluorescence/absorbance each hour for the first 6-8 hours, and at 24 hours.

4.Measure absorbance at a wavelength of 570 nm (Reducted - purple) and 600 nm (Oxidized - red).
Or, measure fluorescence with excitation wavelength at 530-560 nm and emission wavelength at 590 nm.

5.Calculate the percent reduction of UptiBlue :
Colorimetric reading: *For optimal result, subtract background OD at 600 nm from OD at 570 nm:*
Calculate OD570-OD600 for each sample, and plot a standard curve to identify the optimal cell concentration for your assay.
Fluorimetric reading: Calculate fluorescence signal from each sample deducted by background fluorescence from the background control.

$$\% \text{ Reduced} = \frac{\text{Conc.}_{\text{RED Test Well}}}{\text{Conc.}_{\text{OX Negative Control Well}}} = \frac{(\varepsilon_{ox})\lambda_2 A\lambda_1 - (\varepsilon_{ox})\lambda_2 A\lambda_2}{(\varepsilon_{RED})\lambda_1 A'\lambda_2 - (\varepsilon_{RED})\lambda_2 A'\lambda_1} \times 100$$

ε_{ox} = molar extinction coefficient of UptiBlue oxidized form (BLUE); ε_{RED} = molar extinction coefficient of UptiBlue reduced form (RED)

A = absorbance of test wells
A'= absorbance of negative control well. The negative control well should contain media + UptiBlue but no cells.
λ_1 = 570 nm (540 nm may also be used).
λ_2 = 600 nm (630 may also be used).

6.plot the log of cell density (x-axis) versus and the reduction of UptiBlue (calculated from absorbances) or fluorescence (y-axis). Determine the optimal cell concentration for the desired incubation time: in the higher range of the linear respons curve if you study an inhibition of growth, or in the lower range if you want to asses a growth.
7.plot the number of hours incubated (x-axis) versus the reduction of UptiBlue (calculated from absorbances) (y-axis). This can be used to study the kinetic of growth or of a toxic effect, or to determine the maximum incubation time, in which the control cells turn the indicator from the oxidized (blue) form to the fully reduced (red) form.

2-Example Procedures - Adherent cells assays - Cytotoxic assays

Preparation of adherent cells for Testing

1.Harvest an appropriate cell line by trypsinization and subsequent trypsin inhibitor treatment. Use of Accutase reagent UPN68081 (see related products), a mild dissociating agent, can improve you results.

2.Centrifuge cells, resuspend in growth medium and count.

3.Calculate the total cell number and adjust to 1 x 10^4 cell/ml. This density should be adapted to each application.

4.Add 250 µl of cell suspension to each well. Incubate at 37° in 5% CO_2 atmosphere for the number of days required for the particular cell line to be in log phase (usually 3 days).

Exposing Cells to Test Agents – Cytotoxic assays

1.Prepare appropriate dilutions of test agent in growth media.

2.Aspirate spent growth medium from the wells and add 250µl of each dilution of test agent to the wells.

3.Cover, then return to the incubator for 2 days.

4.After incubation, add 25 µl of the indicator to each well. Incubate panels for an additional 3 hours. Panels may then be read spectro**photo**metrically (absorbance at 570 nm and 600 nm) or spectro**fluo**rometrically (excitation : 530-560 nm ; emission : 590 nm).

Data Analysis: Fluorescence :

1.Calculate percent of fluorescence (fluorescence 590nm of test agent sample divided by fluorescence of untreated control).

2.Plot the percent of fluorescence for a given test agent (y-axis) versus the concentration of the test agent (x-asis).

3.Determine the LD_{50} endpoint from the graph by reading from where the 50 percent point intercepts the Dose Response Curve to the concentration along the x-axis. That concentration is the LD_{50} value.

Data Analysis: Absorbance :

Calculate percent of absorbance – ratiometric method
* To determine the percent reduction of UptiBlue:

e. $$\% \text{ Reduced} = \frac{(\varepsilon_{ox})_{\lambda 2} A_{\lambda 1} - (\varepsilon_{ox})_{\lambda 1} A_{\lambda 2}}{(\varepsilon_{RED})_{\lambda 1} A'_{\lambda 2} - (\varepsilon_{RED})_{\lambda 2} A'_{\lambda 1}} \times 100$$

* To calculate the percent difference in reduction between treated and control cells in cytotoxicity/proliferation assays:

f. $$\% \text{ Reduced} = \frac{(\varepsilon_{OX})_{\lambda 2} A_{\lambda 1} - (\varepsilon_{ox})_{\lambda 1} A_{\lambda 2} \text{ of tests agent dilution}}{(\varepsilon_{OX})_{\lambda 2} A^{\circ}_{\lambda 1} - (\varepsilon_{OX})_{\lambda 1} A^{\circ}_{\lambda 2} \text{ of untreated positive growth control}} \times 100$$

Where
C_{RED} = concentration of reduced form UptiBlue (RED)
C_{OX} = oxidized form of UptiBlue (BLUE)
ε_{OX} = molar extinction coefficient of UptiBlue oxidized form (BLUE)
ε_{RED} = molar extinction coefficient of UptiBlue reduced form (RED)
A = absorbance of test wells
A' = absorbance of negative control well. The negative control well should contain media + UptiBlue but no cells.
A° = absorbance of positive growth control well
λ_1 = 570nm (540nm may also be used)
$\lambda 2$ = 600nm (630 may also be used)

Table of the necessary values for solving the equations stated above:

Wavelength (λ)	ε_{RED}	ε_{OX}
540nm	104,395	47,619
570nm	155,677	80,586
600nm	14,652	117,216
630nm	5,494	34,798

1.plot a semi-log graph with the percent of absorbance for a given test agent (y-axis) versus the concentration of test agent (x-axis)

2.Determine the LD_{50} endpoint from the graph by reading from where the 50 percent point intercepts the Dose Response Curve to the concentration along the x-axis.

Blanking of the plate reader should be done with a well containing media only.

Scientific information

Background

The UptiBlue Viable Cell Counting Assay incorporates a fluorometric/colorimetric growth indicator based on detection of metabolic activity. Specifically, the system incorporates an oxidation-reduction (REDOX) indicator that both fluoresces and changes colour in response to chemical reduction of growth medium resulting from cell growth.

The specific REDOX indicator incorporated into UptiBlue has been carefully selected because of several properties (fluorometric/colorimetric). First, the REDOX indicator exhibits **both fluorescence and colorimetric** change in the appropriate oxidation reduction range relating to cellular metabolic reduction. Second, the REDOX indicator is demonstrated to be **minimally toxic** to living cells. The REDOX indicator has no current or past indication of carcinogenic capacity. Third, the REDOX indicator produces **a clear, stable distinct change** which is easy to interpret.

FT-669412

As cells being tested grow, innate metabolic activity results in a chemical reduction of UptiBlue . Continued growth maintains a reduced environment while inhibition of growth maintains an oxidized environment. Reduction related to growth causes the REDOX indicator to change from oxidized (non-fluorescent, blue) form to reduced (fluorescent, red) form.

Experiments suggest that reduction of UptiBlue requires uptake by the cells.

To test this hypothesis, we grew A549 cells to confluency in T25 flasks using RPMI 1640. The media was then removed from 2 flasks containing cells and replaced with fresh media. Fresh media was also added to a sterile flask containing no cells to serve as a negative control. All flasks were then re-incubated at 37°C, 5% CO_2 for 4 hours.

At the end of the 4 hour incubation, UptiBlue was added to each flask. There was no immediate color change in any flasks upon addition. In one of the flasks containing cells, the media was left in contact with the cell layer, while the other T flask was turned over so that the media was not in contact with the cell layer. All flasks were then incubated for 1 hour at 37° and rechecked for color change.

If UptiBlue reduction occurred simply from the reduction of the external medium, we would expect the flask in which the media was in contact which the cells and the flask in which media was no longer in contact with the cells to exhibit the same amount of reduction. This was not the case. The flask where the media was not in contact with the monolayer following addition of UptiBlue displayed no color change from the blue of the negative control flask. The flask where the cells were in contact with the monolayer was very pink, indicating a higher percentage of reduction. This seems to indicate uptake by the cells is required for reduction of UptiBlue .

After all UptiBlue is converted to a pink and fluorescent compound, it can be further reduced to a colorless and nonfluorescent compound, hence the assay signal decreases even with increased number of cells. Therefore, it is important to conduct a cell number titration assay for each particular cell line of your interest to identify the optimal number of cells for your assay to avoid this potential problem. See protocol for determining the cell plating density.

Compatibility of UptiBlue assay with different components in assay buffer: The presence of 10% fetal bovine serum in the cell culture medium has no effect on the spectrophotometric results; however it causes some quenching of fluorescence (therefore, controls and correction are needed).
The presence of the pH indicator phenol red only minimally interferes (ca. 0.03%) with the assay.
Generally, non-reducing media should be used such as RPMI 1640, Hank's modified Eagle medium, or Dulbecco's modified Eagle medium.

UptiBlue **can be multiplexed** with several chemiuluminescent assays, such a s capase assays to measure apoptosis, or reporter assays to measure a gene or a protein expression.

References

.Ahmed, S.A., et al (1994) A new rapid and non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H] thymidine incorporation assay. J Immunol Meth 170:211-224.
.Fields, R.D. and M.V. Lancaster (1993) Dual-attribute continuous monitoring of cells proliferation/cytotoxicity. American Biotechnology Laboratory 11(4): 48-50.
.Goegan. P., et al (1995) Effects of serum protein and colloid on the UptiBlue assay in cell cultures. Toxic In Vitro 9: 257-266.
.Ishiyama, M., et al (1996) A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, Neutral Red and crystal violet. Biol. Pharm. Bull. 19(11):1518-1520.
.Lancaster, M.V. and R.D. Fields (1992) User defined, colorimetric antimicrobial susceptibility assays. American Clinical Laboratories, April 1992.
.Nociari, M.M., et al (1998) A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. J Immunol Meth 213(2): 157-167.
.Pagé, B., et al (1993) A new fluorimetric assay for cytotoxicity measurements in vitro. Intl J Oncol 3: 473-479.
.Woller G. *et al*., Platelet factor 4/CXCL4-stimulated human monocytes induce apoptosis in endothelial cells by the release of oxygen radicals, *Journal of Leukocyte Biology* (2008) Article

Other information

Related products and documents:

NT-66941f:	additional technical documents with complete values and calculation. (effect of different buffer, phenol red, plate storage, temperature,...)
NT-66941a:	Applications review
UPN68081	Accutase reagent (mild dissociating agent – replaces advantageously trypsinization)
Ask	ATP assays, Caspase assays, Reporter assays Cell culture media and components

rev. I09E-H01E-B09E