

UptiBlue

Product Description

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.

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

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

UptiBlue is supplied as a sterile indigo coloured liquid.

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

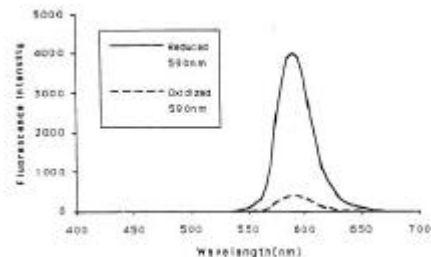
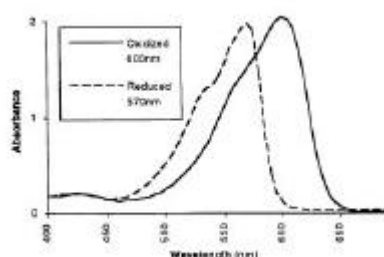
Features	Benefits
Fluorescent/Colorimetric Fluorescence is monitored at 530-560nm excitation wavelength and 590 nm emission wavelength	.Allows choice of detection method : either with a spectrophotometer or a spectrofluorometer .no interference from the presence of 10% fetal bovine serum, nor from phenol 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

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.

Contact your local distributor

Uptima, powered by



Uptima@interchim.com

Directions for Use

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

1. Harvest cells which 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.
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. Measure absorbance at a wavelength of 570 nm and 600 nm. Or, measure fluorescence with excitation wavelength at 530-560 nm and emission wavelength at 590 nm.

4. calculate the percent reduction of UptiBlue :

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

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

5. 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.
6. 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.

Example Procedure of Cytotoxicity assay

Preparation of cells for Testing

1. Harvest an appropriate cell line by trypsinization and subsequent trypsin inhibitor treatment.
2. Centrifuge cells, resuspend in growth medium and count.
3. Calculate the total cell number and adjust to 1×10^4 cell/ml. This density should be adapted to each application.
4. Add 250 μ l of cell suspension to each well. Incubate at 37° in 5% CO₂ 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

1. Prepare appropriate dilutions of test agent in growth media.
2. Aspirate spent growth medium from the wells and add 250 μ l of each dilution of test agent to the wells.
3. Cover, then return to the incubator for 2 days.
4. After incubation, add 25 μ l of the indicator to each well. Incubate panels for an additional 3 hours. Panels may then be read spectrophotometrically (absorbance at 570 nm and 600 nm) or spectrofluorometrically (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-axis).
3. Determine the LD₅₀ 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 LD₅₀ value.

Data Analysis: Absorbance :

1. Calculate percent of absorbance with the following formula :

$$= \frac{(\epsilon_{\text{OX}})\lambda_2 A\lambda_1 - (\epsilon_{\text{OX}})\lambda_2 A\lambda_2 \text{ of test agent dilution}}{(\epsilon_{\text{OX}})\lambda_2 A\lambda_1 - (\epsilon_{\text{OX}})\lambda_2 A\lambda_2 \text{ of untreated positive growth control}} \times 100$$
2. 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)
3. Determine the LD₅₀ endpoint from the graph by reading from where the 50 percent point intercepts the Dose Response Curve to the concentration along the x-axis.

Scientificad Technical Information

Scientific background

The UptiBlue Viable Cell Counting Assay incorporates a fluoro metric/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 (fluorometric/colorimetric) REDOX indicator incorporated into UptiBlue has been carefully selected because of several properties. 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. Third, the REDOX indicator produces a clear, stable distinct change which is easy to interpret. The REDOX indicator has no current or past indication of carcinogenic capacity.

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% CO₂ 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 with 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 .

Literature

- .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 alamarBlue 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.

Other Information

An additional technical document #DT-UP66914 with complete values and calculation is available.

For Research Use Only. Not for Use in Diagnostic Procedures. It is not to be used in humans or animals. There is no express or implied warranty. No liability is assumed for fitness of purpose and merchantability, or direct and consequential damage. The user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with all regulations.

For any question, please ask Uptima or your local distributor.

rev. B09E

Contact your local distributor

Uptima, powered by



213 Avenue J.F. Kennedy - BP 1140
93163 Montreuil Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 86

Uptima@interchim.com