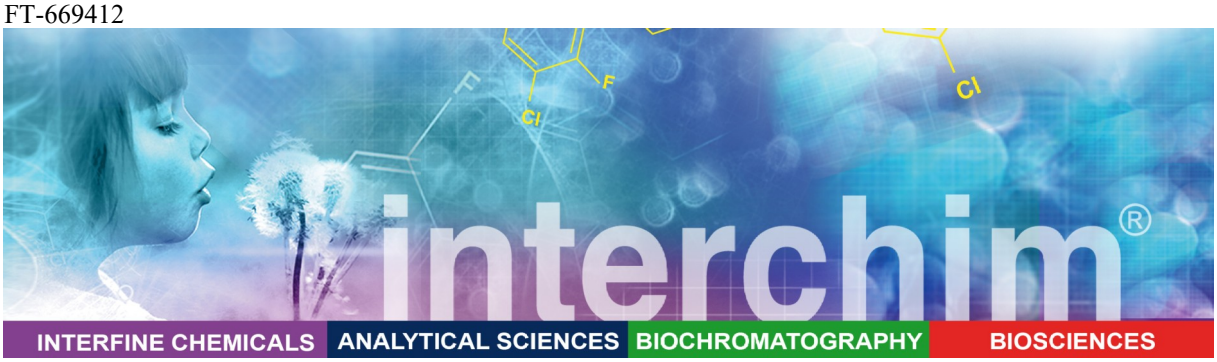
FT-669412



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 [^{3}H] thymidine assay[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 : +4°C (H)(L)
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**
Colorimetric : read with 570nm and 600nm filters Fluorescent : $\lambda_{exc//em}$: **570 / 590 nm**	.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

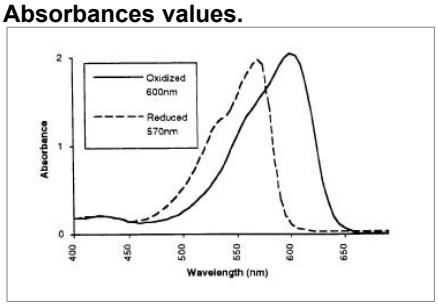
UptiBlue™ is supplied as a sterile indigo coloured liquid.

Control of UptiBlue reagent. Absorbances values.

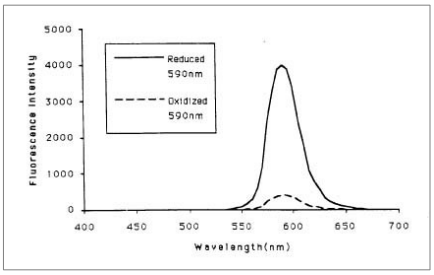
In Uptima standard conditions*, absorbances values are :

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



absorbtion



emission

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 : Fluorescence units are arbitrary and the scale used varies widely from one instrument to another.

Directions for Use

Note: L'UptiBlue™ can be further reduced to hydroresorufin (colorless and non-fluorescent). At higher cell densities or with prolonged development times, the assay signal can initially increase, then decrease after all UptiBlue is converted into resorufin, which then begins to be further reduced to hydroresorufin. Therefore, it is important to conduct a cell number titration (standard curve) to identify the optimal plating density and development time that generates signal that increases proportionally with cell number.

1. Plate cells in 96-well tissue culture plates in 100 µL/well. For a standard curve, plate a series of cell dilutions in the range of 40-20,000 cells per well for adherent cells, and 2,000 to 500,000 cells per well for suspension cells. For fluorescence-based detection, include a well with 100 µL of cell culture medium without cells to use as a background control.
2. After cells have reached the desired density, add 10 µL UptiBlue solution to the medium in each well, and mix thoroughly.
3. Incubate the plate for between 1 hour and 24 hours at 37°C.

Note: Signal from the same plate can be read at multiple time points to determine the optimal incubation time for your cell type and density.

4. For colorimetric detection, measure absorbance at 570 nm and 600 nm using an absorbance microplate reader. For fluorescence-based detection, measure fluorescence with excitation/emission at 570/590 nm using a fluorescence microplate reader.

Note: Fluorescence-based detection is more sensitive and has broader dynamic range than colorimetric detection.

Note: The excitation and emission spectra of resorufin are fairly broad, excitation filters between 530–570 nm and emission filters between 580-620 nm can be used.

5. For the colorimetric detection method, subtract background absorbance at 600 nm from resorufin absorbance at 570 nm. For fluorescence-based detection, subtract fluorescence at 590 nm from the background control (culture medium without cells) from each cell sample.
6. Plot cell plating density vs. background-subtracted absorbance or fluorescence for your cell number titration to determine the optimal assay conditions for your cell line.

Scientific information

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 for its great properties. First, the REDOX indicator exhibits **both fluorescence and colorimetric** change in the appropriate oxido-reduction status change relating to cellular metabolism. Fluorescence especially achieves very **high sensitivity**. 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.

As cells being tested grow, innate metabolic activity results in a chemical reduction of UptiBlue™, that changes from oxidized (non-fluorescent, blue) form to reduced (fluorescent, red) form. Continued growth maintains a reduced environment while inhibition of growth maintains an oxidized environment.

UptiBlue™ does not stains the cells, however experiments suggest that reduction of UptiBlue™ requires uptake by the cells.

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 chemiluminescent assays, such as cytokine assays, caspase assays to measure apoptosis, or reporter assays to measure a gene or a protein expression.

The **toxicity** of UptiBlue™ is so low, so that, after the Cell Counting assay is completed, the same cells can be used for other assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay.

If you do not have a 450 nm filter, you can use filters with the absorbance between 450 and 490 nm, even though 450 nm gives the best sensitivity.

More informations in the technical notice 66941a (for applications) and 66941f (technical background).

Troubleshooting Questions

Q: Why are the fluorescence values lower compared to previous assays ?
A: The resazurin solution can sometimes precipitate out solid resazurin. This results in a lower concentration and lower fluorescence. Heat the UptiBlue™ solution at 37 C for 2 hours and the solid will go back into solution.

References

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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

PH-BA362a FPlyte microplates (for fluorescence assays – low autofluorescence),
i.e. 96 x 350µl-wells Black/clear bottom #FP-BA7890 (or FP-BA7990 for NTC),
384 x 120µl-wells Black/clear bottom #FP-BA8290 (or #FP-BA8170 for MCT, or #FP-BB1450 for 30µl wells)

UPN68081 Accutase reagent (mild dissociating agent – replaces advantageously trypsinization)

Ask related products : ATP assays, Caspase assays, Reporter assays ; Cell culture media and components ; ...

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