# Cell counting, viability & proliferation

Cell counting is required

- To monitor cells during cell cultures
- For cell preparation or any cell experiment
- To standardize cell samples for analysis.
- Cell proliferation
- Cytotoxicity assays

Several methods have been proposed, each fitting more or less to each specific application : counting dead cells may be acceptable for the preparation of cell extracts or desired when one do not want to operate with hazardous cells or for cytotoxicity study. At the opposite dead cells counting is generally precluded for cell culture and bioassays. It may be useful to quantitate only viable cells, or only fast proliferating cells.

Interchim provides a large choice of cell assays covering standard as well as innovative methods for general to specific cell assays.

## Technical tip

**MicroPlate readers & Imaging systems**

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with instruments.

BERTHOLD TECHNOLOGIES

***NightOWL LB983 NC100**

***Mithras LB940 MultiMode Reader**

## Selection guide

| Probe | Principle | Detection Method | Dead | Viable | Proliferating | Features/Advantages - Drawbacks |
|---|---|---|---|---|---|---|
| **Trypan blue** | Membrane exclusion | Colorimetric Microscopy | ++ | ++ | ++ | Cheap, but time consuming, not scalable. Do not state on viability. |
| **Hoechst** | DNA probe exclusion | Fluorimetric | ++ | ++ | +++ | Cheap, Scalable, Non toxic. Do not state on viability. More rapid than MTT/XTT ; unfixed or fixed samples. |
| **MTT** | Formazan dye, orange precipitate. | Colorimetric | - | ++ | +++ | Popular method. Sensitive, Scalable. Non toxic Increased solubility and performance from MTT to XTT and WST. |
| **XTT** | Same as MTT but more soluble. | Colorimetric | - | ++ | +++ | |
| **WST** | Formazan dye, soluble & not toxic | | - | ++ | +++ | |
| **UptiBlue** | ratiometric blue probe for cell redox | Colorimetric Fluorimetric | - | +++ | +++ | No solubilization step (unlike MTT). Applyalso to adherent cells. Sensitivity similar to MTT/XTT, but easier to use Fluorimetry/Superior sensitivity to MTT / XTT. |
| **Calcein-AM** | Calcein accumulation in cytoplasm | Fluorimetric | - | +++ | ++ | No solubilization step (unlike MTT/XTT). Adaptable to a wide variety of techniques, including : microplate assays, in vivo cell tracing. Do not work for bacteria. May alter some cell functions. |
| **GAPDH** | Release of GAPDH coupled to ATP assay | Bioluminescence | - | +++ | + | Measurement of Cell-Mediated (T Cells, ADCC, NK) or Complement-Mediated Cytolsis. |
| **CFSE** | Fluorescein protein labeling | Fluorimetric | ++ | ++ | ++ | Useful when other method do not work properly. Do not state on viability. |
| **AnnexinV** | AnnexinV/PhosphoSerine | Fluorimetric | + | +++ | + | Useful for Apoptosis study. |
| **LDH** | convertion in colored product | | - | ++ | + | Recommended for cytotoxicity assays Serum Interference. |
| **Luciferin Syst.** | ATP measure | Luminescence | - | + | +++ | Pros : sensitivity / linearity. Cons : signal depends on each cell line, on temperature |
| **-3H Thymidine** | DNA incorporation of radioactivity | Radioactivity | - | + | +++ | Cons : hazardous (radioelements). |
| **BRDU** | DNA incorporation | Immunoassay | - | + | +++ | |
| **$^{51}$Cr release EU$^{3+}$** | Release of radioactivity by cytoplasm | Radioactivity | - | - | +++ | Recommended for cytotoxicity assays. Cons : hazardous (radioelements). |
| **Propidium Iodide, AAD** | **7-**Membrane permeability | Fluorimetric | +++ | - | - | Used in combinaison of green fluorescence dye like. Annexin V-FP488 to discriminate dead cells from alive cells. |

# Dual staining to detect live and dead cells

## ■ Live/Dead Mammalian Viability Assay Kit

Two-color fluorescent staining of live (green) and dead cells (red)

- Dual Detection : Detect both live and dead cells simultaneously.
- Simple & Fast : Require only a 30-min dye loading time and then measure without washing.
- Economical : Perform viability and cytotoxicity assays at the same time.
- Versatile : Analyze with flow cytometers, fluorescence microscopes or fluorescence plate readers.

The Viability/Cytotoxicity Assay Kit for Live / Dead Cells provides a two-color fluorescence staining on both live and dead cells using two probes that measure two recognized parameters of cell viability — intracellular esterase activity and plasma membrane integrity [Papadopoulos, 1994]. The kit is suitable for use with fluorescence microscopes, fluorescence multiwell plate scanners and flow cytometers and other fluorescence detection systems. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells [Vaughan, 1995] and certain tissues [Poole, 1993], but not to bacteria or yeast. This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, $^{51}Cr$ release and similar methods for determining cell viability and cytotoxicity. It is generally faster, less expensive, safer and a more sensitive indicator of cytotoxic events than alternative methods. EthD-III shares the same property with EthD-I used in Live/Dead Viability/Cytotoxicity Assay Kit #486301 and is 40% brighter at intensity compared to EthD-I. Validity of the Live/Dead Viability/Cytotoxicity assay for animal cell applications has been established by several publications.

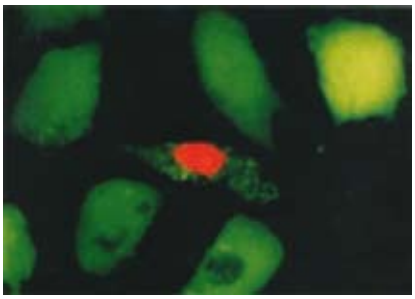
Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-III enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-III is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually nonfluorescent before interacting with cells.

If cells are first fixed, and then stained, the Live/Dead Bacterial Viability/Cytotoxicity kit can also be considered. To replace the dye Calcein AM that will only stain the live cells, the DMAO; a DNA-binding dye, will stain both intact and damaged cell membranes.

**References :**

J Immunol Methods, 177, 101 (1994).
J Cell Sci, **106**, 685 (1993).
J Neurosci, **15**, 5389 (1995).

| Description | P/N : | Qty |
|---|---|---|
| Live/Dead Mammalian Viability Assay Kit | **FP-BF4710** | 1000 tests in microplate reader |
| **Related products :** | | |
| DMAO, nuclei stain for live cells, 2 mM soln in DMSO | **FP-CA8150** | 1 ml |
| Ethidium Bromide III, 1 mM solution | **FP-BP9341** | 200 µl |
| MTT ($\lambda_{abs.}$(cleaved) : 650 nm (550-600 nm)) | **FP-65939A** | 1 g |
| Live/Dead Yeast Viability Assay Kit based on calcein-AM and PI. | **486301** | 1 kit |

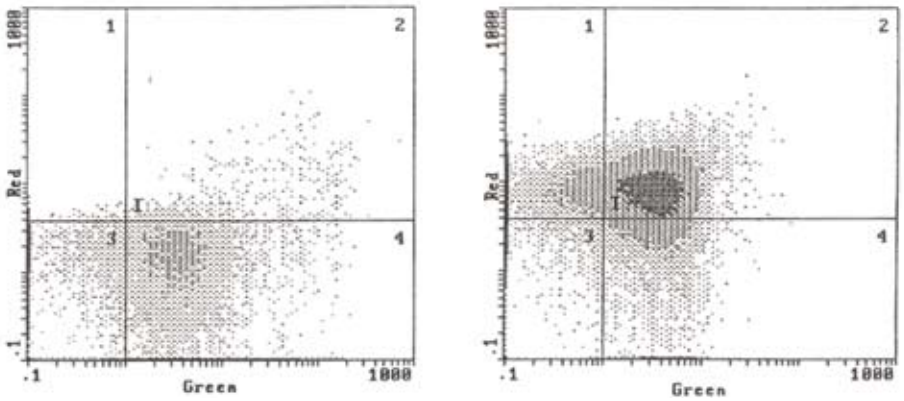


Hela Cells incubated with assay solution.

## Live/Dead Bacterial Viability/Cytotoxicity kit

Two color fluorescence staining on both live bacteria (green) and dead bacteria (red)

- Dual Detection : Detect live and dead bacteria cells in a cell population simultaneously.
- Simple & Fast : 15 min dye loading and measure without washing.
- Economic : Perform viability and cytotoxicity assays at the same time.
- Versatile : Analysis compatiable with flow cytometers and fluorescence microscopes using popular settings for fluorescein and propidium iodide.




Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cell Staining Kit provides two-color fluorescence staining on both live (green) and dead (red) bacteria using two probes, DMAO and EtD-III. DMAO is a green-fluorescent nucleic acid dye that stains both live and dead bacteria with intact and damaged cell membranes. EtD-III is a red-fluorescent nucleic acid dye that stains only dead bacteria with damaged cell membranes. With an appropriate mixture of DMAO and EtD-III, bacteria with intact cell membranes is stained fluorescent green, whereas bacteria with damaged cell membranes is stained fluorescent red. The kit is suitable for use with fluorescence microscopes and flow cytometers. The assay principles are general and applicable to most bacteria types.

A common criterion for bacterial viability is the ability of a bacterium to reproduce in suitable nutrient media that is referred to as growth assays. This kit yields results that correlate well with growth assays in liquid or solid media. Under certain conditions, however, bacteria having damaged membranes may be able to recover and reproduce — such bacteria may be scored as "dead" in this assay. Conversely, some bacteria with intact membranes may be unable to reproduce in nutrient medium, and yet these may be scored as "alive". Therefore, these situations need to be considered if a vast difference of live and dead bacteria counts is observed between this assay and growth assays.

This kit can also be considered if cells like mammalian cells, are first fixed, and then stained.

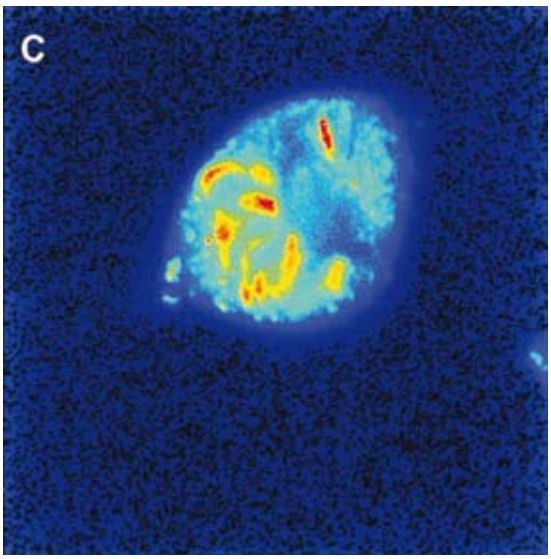
| Description | P/N : | Qty |
|---|---|---|
| Live/Dead Bacterial Viability/Cytotoxicity | **FP-BU1040** | 1000 tests in microplate reader |
| **Related products :** | | |
| Live/Dead Yeast Viability Assay Kit<br>based on WST-8 formazan dye. Read at 450 nm (450-490 nm) | **486301** | 1 Kit |

# Dual staining to detect live and dead cells

## ■ Ethidium monoazide, bromide (EMA)

Selectively and covalently labels membrane-damaged or metabolically compromised cells in the presence of live cells

Ethidium monoazide bromide is a red fluorescent nucleic acid stain with a photoaffinity label. The dye, after photolysis, binds covalently to nucleic acids.[1] After photocrosslinking to DNA, the wavelengths ($\lambda_{exc.}$ / $\lambda_{em.}$ = 504 / 600 nm) are compatible with a simultaneous observation of another green indicator. The dye has been used to "footprint" drug binding sites on DNA[2] to modify plasmid DNA,[3,4] and to determine hemopoietic cell phenotype, function and position in the cell cycle.[5] A particularly useful application of the dye is to selectively and covalently label dead cells in the presence of live cells. Since ethidium monoazide bromide is relatively impermeant to live cells, it selectively labels DNA in dead cells in a mixed population of live and dead cells. Photolysis following the dye application renders the dead cell DNA covalently labeled with the dye. One can then wash and fix the cell preparation and exam it by microscopy fluorescence plate reader or flow cytometry. The major advantage of this method is that researchers can avoid extensive manipulation of live pathogenic organisms.[6] At the difference of propidium iodide, the ethidium monoazide binds covalently, and, when applied to cells before fixation, provides an indication of what fraction of the unfixed population were membrane-damaged or metabolically compromised.




Two photon fluorescent image of live PTK2 cells vitally stained with EMA : A late prometaphase cell illustrating the high selectivity of the stain for the chromosomes.

**References :**
1)J. Mol. Biol. 92, 319(1975)
2) Euro. J. Biochem. 182, 437(1989)
3)J. Biol. Chem. 257, 13205(1982)
4) J. Biol. Chem. 259, 11090(1984)
5) Cytometry 11, 610(1990)
6)Cytometry, 12, 133(1991)
7) PNAS, 97 , no. 17, p. 9504-9507 (2000)

| Description | P/N : | Qty |
|---|---|---|
| Ethidium monoazide, bromide (EMA)<br>$\lambda_{ex.}/\lambda_{em.}$(DNA bound) : 504/600 nm ; MW : 420.3 | **FP-48256A** | 5 mg |

## ■ Propidium monoazide (PMA)

Selectively and covalently labels dead cells in the presence of live cells

PMA™ is a derivative of EMA, but it has significantly higher DNA binding affinity and is cell impermeant. As EMA, after photolysis, the dye is converted to a fluorescent DNA stain covalently bound to DNA.

| Description | P/N : | Qty |
|---|---|---|
| Propidium monoazide (PMA)<br>$\lambda_{ex.}/\lambda_{em.}$(DNA bound) : 510/610 nm ; MW : 512 | **FP-BZ9340** | 1 mg |

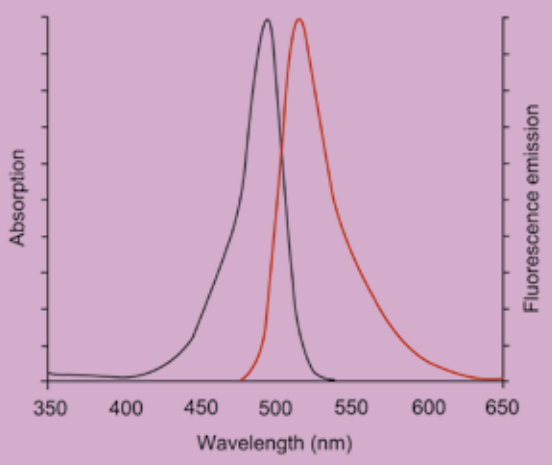
**References :**
**Nocker, A.** *et al.,* Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. *Microbio Meth. 67(2)*, 310-320 (2006).
**Nocker A.** *et al.,* Use of Propidium Monoazide for Live/Dead Distinction in Microbial Ecology, *Applied and Environmental Microbiology*, p. 5111-5117, Vol. 73, No. 16 (2007).
**Pan Y., Breidt F.,** Enumeration of *Listeria monocytogenes* by Real-Time PCR with Propidium Monoazide and Ethidium Monoazide in the Presence of Dead Cells, *Appl. Environ. Microbiol.* doi:10.1128/AEM.01198-07 (2007).




## Technical tip

**Calcein** dye is a polyanionic derivate of fluorescein that exhibits fluorescence that is essentially independent of pH between 6.5 and 12. The excitation and the emission wavelengths of calcein are 485 nm and 535 nm, respectively. It is well retained in cells. These features have made it a popular and versatile dye for various applications, including cell volume changes in neurons and other cells, endocytosis, gap junctional communication, membrane integrity and permeability, angiography, liposomes…
It is worthy to notice that calcein is strongly quenched by several ions, including $Fe^{3+}$, $Co^{2+}$, $Cu^{2+}$ and $Mn^{2+}$ at physiological pH (not by $Ca^{2+}$ or $Mg^{2+}$ ions). Ions levels should thus be monitored.

**AM ester** is membrane-permeant and enters readily cell membranes. Intracellular esterases convert it into calcein. The DMSO solution is more convenient (time saving, reduces solubilization variability) especially for more reproductible screening assays.




Fluorescence of calcein at pH9.0

## ■ Calcein AM Cell Counting & Viability Assay Kit

The Calcein-AM Kit provides a simple, rapid and accurate method to measure cell viability and/or cytotoxicity. The kit utilizes calcein AM for the fluorometric determination of living cell numbers. The amount of a fluorescent dye read at 512 nm, calcein, hydrolyzed by esterases in cells, is directly proportional to the number of viable cells in culture media. The 96-well microplate assay has a detection range of less than 50 cells to more than 25 000 cells per well. It can be used for 384-well plates by adding 5 µl (instead of 10 µl) assay solution to 50 µl PBS solution per well. Since esterases and phenol red in the culture medium interfere with the fluorescence measurement, replacing the cell culture medium with PBS is necessary prior to adding the Calcein-AM assay solution. An incubation of 10 to 30 minutes gives sufficient fluorescence intensity for the cell viability determination.

**Features :**

- Suitable for proliferating and non-proliferating cells
- Ideal for both suspension and adherent cells
- Non-radioactive microplate
- Rapid (no solubilization step as in an MTT assay)
- Ideal for high-throughput assays
- Better retention and brightness compared to other fluorescent compounds (i.e. fluorescein)

**Applications :**

- Cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis, cytotoxicity,...
- Microplate assays, immunocytochemistry, flow cytometry, and in vivo cell tracing

| Description | P/N : | Qty |
|---|---|---|
| Cell Counting Kit, calcein-AM based | **876981** | 500 tests |
| | **876982** | 2 x 500 tests |
| Calcein AM | **FP-895514** | 1 mg |
| $\lambda_{ex}/\lambda_{em}$ (cleaved) : 494/517 nm ; MW : 994.9 | **FP-895515** | 20 x 50 µg |
| Calcein AM, 1 mg/ml in anhydrous DMSO | **FP-855422** | 1 ml |
| Calcein AM, 4 mg/ml in anhydrous DMSO | **FP-FI9820** | 100 µl |
| Calcein AM, 5 mM in anhydrous DMSO, Pure Grade | **FP-JQ8140** | 200 µl |
| **Related products :** | | |
| Annexin V-FluoProbes 488, Flow Cytometry Grade (495/519 mm) | **FP-BH9390** | 100 tests |
| Propidium iodide, 1 mg/ml | **FP-36774A** | 10 ml |

## ■ CFDA, SE for microbial and cell enumeration

5-(and 6-)-carboxyfluorescein diacetate, succinimidyl ester (CFDA, SE) efficiently stains gram-negative and gram-positive bacterial genera without causing undesirable effects on cell adhesion or viability.
The high throughput method using microplate spectrofluorometry has a detection limit of mid-$10^5$ CFDA-stained cells/ml.
CFDA, SE tracking technique has applications in bacterial transport, public health microbiology, allowing the movement of pathogen to be monitored in terrestrial, aquatic, and even food-processing environments. The technique may also be useful for studying infection and colonization by pathogens in vivo using animal models.

**Reference :**
Mark F. et al. - Development of a Vital Fluorescent Staining Method for Monitoring Bacterial Transport in Subsurface Environments, Applied and Environmental Microbiology, October 2000, p. 4486-4496, Vol. 66, No. 10

| Description | P/N : | Qty |
|---|---|---|
| CFDA-SE (CFSE, Green Cell Tracking reagent)<br>$\lambda_{ex./em.}$ (cleaved) : 495/519 nm ; MW : 557 | **FP-52493A** | 25 mg |

## ■ UptiBlue Cell Viability Assay Kit

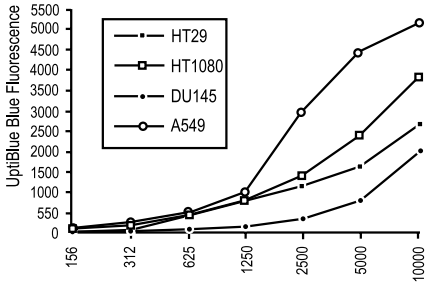
Substrate : Resazurin
$\lambda_{ex./em.}$ : 540 / 590 nm
Sensitive : 100 cells

Principle : the UptiBlue dye enters readily into cells, where it elicits a wavelength shift of absorbance and a strong fluorescence related to redox potential in cell, informing on cell energetic state.

UptiBlue shows excellent correlation to formazan and tritiated thymidine techniques, while being much easier and safer to use. It especially replaces advantagely MTT/XTT in many applications, from cell counting to proliferation assay and cytotoxicity testing. Furthermore it allows longer studies.

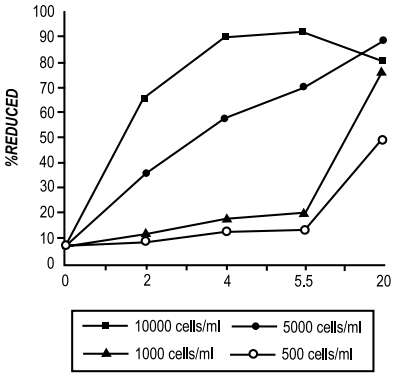
**Applications**

**Cell proliferation assay**



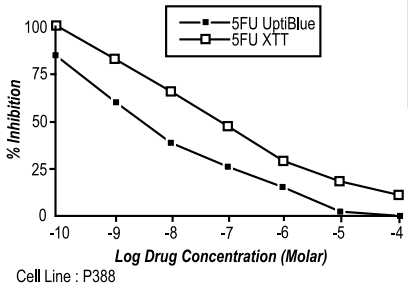

Detection of cell Growth of 4 Cell Lines using UptiBlue

**Kinetic / long term assays**




Kinetic reduction curve with UptiBlue with plating density from 500 to 10000 cells A549/ml.

**Cytotoxicity assay**




Cell Line : P388
Cell density 1250 cells/well, 5 days exposure to5-Fluorouracï
4 hours incubation with UptiBlue or XTT (+ menadione).

Determination of Doxorubicin $LD_{50}$ using UptiBlue and XTT

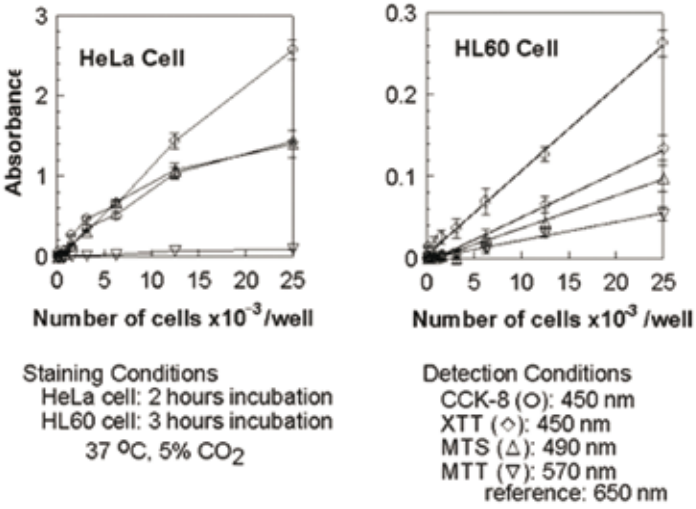
| Description | P/N : | Qty |
|---|---|---|
| UptiBlue Cell Viability Assay Kit | **UP669412** | 25 ml |
| | **UP669413** | 100 ml |

## WST-8 Cell Proliferation and Cytotoxicity Assay Kit

- Colorimetric microplate assay
- **Ready-to-use** one-bottle solution
- **Safe** : no radioisotope or organic solvent required
- **No toxicity** to cells
- **Easy and fast** : no harvesting, washing or solubilization step required
- **More sensitive** than MTT, XTT, MTS and WST-1

Reduced toxicity of assay solution :


Cell proliferation assay :




CCK-8 consists of WST-8 and 1-methoxy PMS as an electron mediator. After the plate is incubated for 1-4 hours in the incubator, the absorbance is measured in 96 or 384-well plate. The wavelength range for the measurement of the absorbance is between 450 nm and 490 nm. The amount of the yellow colored formazan dye generated by dehydrogenases in cells is directly proportional to the number of viable cells in a culture medium. The sensitivity using CCK-8 is higher than that using MTT or the other tetrazolium salts that produce water-soluble formazan dyes such as XTT or MTS for HeLa cells and HL60 cells. Furthermore, the cell proliferation assay data using CCK-8 correlates with that using the $^{3}$H-thymidine incorporate assay.

| Description | P/N : | Qty |
|---|---|---|
| CKK-8 Cell Counting Kit | **899650** | 1000 tests |
| | **899651** | 3000 tests |
| | **899654** | 10000 tests |
| **Also available :** | | |
| MTT Cell Proliferation Assay Kit | **45547A** | 1000 tests |
| MTT UltraPure | **FP-65939A** | 1 g |
| XTT Cell Proliferation Assay Kit | **FX873A** | 1000 tests |
| XTT UltraPure | **FP-409036A** | 1 g |
| WST-1 Cell Proliferation Assay Kit | **KS0790** | 96 tests |
| WST-1 as stand alone product | **F98883** | 100 mg |

### Technical tip

**Formazan based Cell Viability Assay Kit**

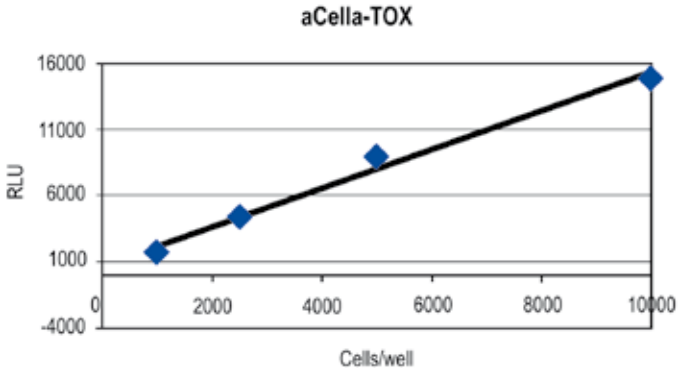
MTT based assay is probably the most popular cell viability assay. It has several drawbacks including toxicity, poor solubility that requires an extraction step and limited sensitivity. Interchim provides those kits as well (see related products) but recommends strongly the WST-8 assay kit, or alternatively the UptiBlue reagent.

## ■ aCella™ - TOX Bioluminescence Cytotoxicity Assay (GAPDH)

Measurement of Cell-Mediated (T Cells, ADCC, NK) or Complement-Mediated Cytolysis

- **Versatile :** Assay can be run in serum supplemented media.
- **Homogenous** - One-step, no wash assay. Assay can be run in same plate as samples.
- **FAST** - Results in 3-5 minutes.
- **Highly Sensitive** - Can detect fewer than 500 cells/well.
- Works with PRIMARY CELLS for determining cell Cytotoxicity.
- **Non-destructive** assay allows monitoring of additional parameters.

aCella-TOX provides a new and highly sensitive assay using a patented coupled luminescent technology for the detection of cytotoxicity[1]. This assay quantitatively measures the release of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) from Primary Cells, mammalian cell lines, bacterial cells[1,2,3]. aCella-TOX can work in different media formulations and allows overnight assays while retaining its sensitivity. The sensitivity of aCella-TOX is also greatly enhanced by the coupled luminescent signal-amplification system (3-Phosphoglyceric Phosphokinase/ATP/Luciferase), which yields a strong luminescent signal from even small amounts of released enzyme.




Jurkat cells were plated at various cell concentrations per well. NP-40 cytotoxic agent was added to each well. The aCella-TOX kit was used to detected G3PDH enzyme release. Data points show average RLU in triplicate.

In the aCella-TOX reaction scheme the release of GAPDH is coupled to the activity of the enzyme 3-Phosphoglyceric Phosphokinase (PGK) to produce ATP. ATP is detected via the luciferase, luciferin Bioluminescence methodology. Further, aCella-TOX is a homogeneous cytotoxicity assay ; alternatively in dual mode, aCella-TOX can measure cytotoxicity and cell viability in the same plate. Culture supernatants can also be removed from the original plate and assayed in a different plate, allowing kinetics runs to be set up. The assay is non-destructive, allowing the monitoring of additional parameters such as gene expression.
The method is highly general, since all known cells express copious amounts of GAPDH, and, unlike other enzymes, GAPDH is very readily released from the cytoplasm upon cell lysis. Using specially adapted formulations, the sensitivity of the method can be driven below 1 eukaryotic cell [2].

**Applications :**
The aCella-TOX method has been tested with many modes of cytolysis, including :

- Cellular cytotoxicity (T cells)
- Complement[2,3], pore-forming agents
- Antibiotic-mediated lysis of bacteria
- Detergent mediated and mechanical lysis

**References :**

1. Methods and compositions for coupled luminescent assays. United States Patent 6,811,990 Corey and Kinders, issued November 2, 2004.
2. Corey, M. J. and Kinders, R. J. (2005), Drug Discovery Handbook, Ed. Shayne Cox Gad, pp. 689-731
3. Corey, M.J., et al Journal of Immunological Methods 207:43-51, 1997.
4. Corey, M. J., et al., Journal of Biological Chemistry 275: 12917-12925, 2000.
5. Ogbomo H., et al. - Biochemical and Biophysical Research Comunications 339 (2006) pp375-379.
6. Corey, J. and Kinders, J. (2005), Drug Discovery Handbook, Ed. Shayne Cox Gad, pp. 689-731

| Description | P/N : | Qty |
|---|---|---|
| aCella-Tox bioluminescent Cytotoxicity Assay | **CA4670** | 500 tests |
| Kit Content : 4x Enzyme Assay Reagent, 1x Enzyme Assay Diluent, Glyeraldehyde 3-Phosphate (G3P), 50x Detection Reagent, 5.5x Detection Assay Diluent, Lytic Agent | | |




## ■ ATP Assay kit, 0.1 to 100 pmol

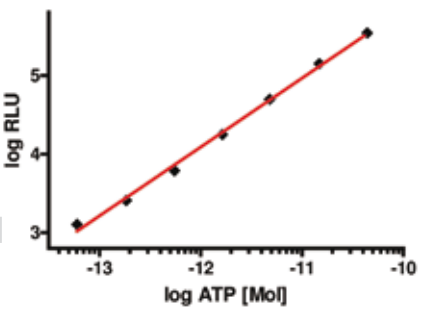
To detect ATP in biological samples or monitore ATP dependent enzyme assays

Substrate : luciferin with stabilizer
$\lambda_{em.}$ : **560 nm**
Sensitivity : **0.1 to 100 pmol ATP** - 30 min. signal

The ATP Determination Kit, sensitive assay, offers a convenient bioluminescense assay for quantitative determination of small amounts of ATP. Catalysed by firefly luciferase the substrate D-luciferin is oxidized in an ATP-dependent process generating chemiluminescence at 560 nm (pH 7.8) :

**luciferin + ATP+$O_2$** → ($Mg^{2+}$, luciferase) → **Oxyluciferin + ATP + pyrophospate + $CO_2$ + light**

The sensitive assay is optimized for fast determination of low levels of pre-existing ATP or ATP formed in kinetic systems. After a 10 min incubation of the assay reagent, ATP concentrations down to 0.1 pmol can be exactly determined using the linear luminescent signal of the luciferase reaction. Loss of luminescent signal and sensitivity is observed after incubation times of more than 30 minutes. If you are interested in a time-stable assay (i.e. for high throughput screenings) with nearly constant luminescence signals over a period of up to four hours, use our Steady Glow ATP Assay Kit.




Linear luminescence signal for ATP concentrations down to 0.1 pmol using the ATP Determination Kit, sensitive assay.

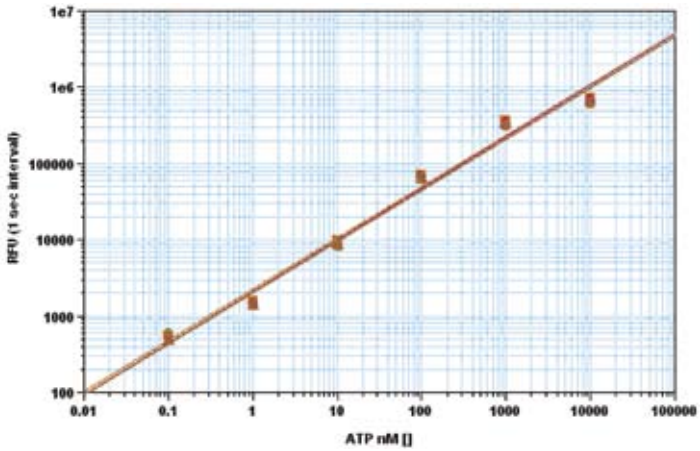
| Description | P/N : | Qty |
|---|---|---|
| ATP Assay Kit, 0.1 to 100 pmol sensitive | **FP-S2841A** | 200-1000 assays (10 ml) |
| Each kit contains : | **FP-S2841B** | 600-3000 assays (30 ml) |
| Component A : Firefly Luciferase (ready to use glycerol stock solution) | **FP-S2841C** | 2000-10000 assays (100 ml) |
| Component B : D-Luciferin (to dissolve in reaction buffer) | | |
| Component C : Dithiothreitol DTT (to dissolve in reaction buffer) | | |
| Component D : Reaction Buffer (ready to use buffer) | | |

**Related products :**

| | | |
|---|---|---|
| ATP disodium salt | **00064A** | 25 g |
| Reaction Buffer as stand alone product (Component D of ATP Assay Kit) | **CA3920** | 30 ml |
| Reaction Buffer as stand alone product (Component D of ATP Assay Kit) | **CA3921** | 100 ml |
| ARL-67156 Ecto-ATPase inhibitor | **CG2331** | 10 mg |

## ■ ATP Assay Kit, Steady Glow

Substrate : luciferin with stabilizer
$\lambda_{em.}$ : **560 nm**
Sensitivity : **10 cells/well** - 10 uM to 0.1 nM ATP - 4 h signal




Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The ATP Assay Kit provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods. This ATP Assay Kit has the stable luminescence signal as long as 4 hours. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

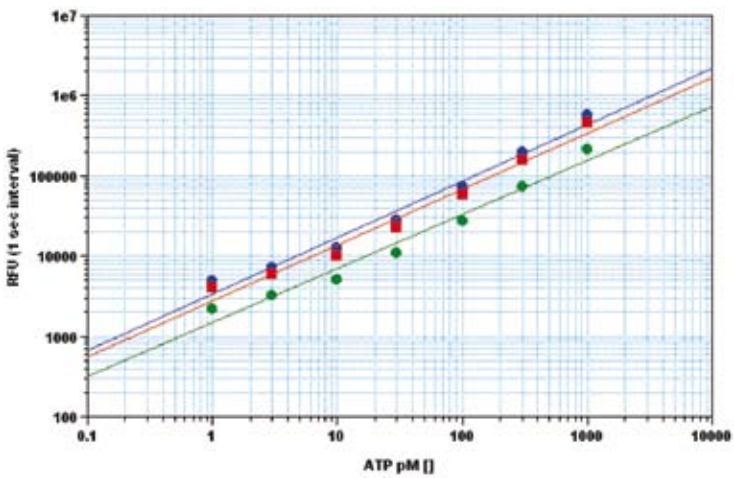
Linear luminescence signal for ATP concentrations from 10 µM to 0.1 nM was detected up to 5 h (Z' factor = 0.7) without signal decayed (above fig shows 20 min, 1, 2, 3, 4, and 5 hr signal). The integrated time was 1 sec.

| Description | P/N : | Qty |
|---|---|---|
| ATP Assay Kit, Steady Glow | **FN0630** | 96 assays |

## ATP Assay Kit, Bright Glow

Substrate : luciferin with stabilizer
$\lambda_{em.}$ : **560 nm**
Sensitivity : **10 cells/well** - 3 pmol ATP 2 h incubation time

Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The ATP Assay Kit provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods. This ATP Assay Kit can detect as low as 10 cells/well. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

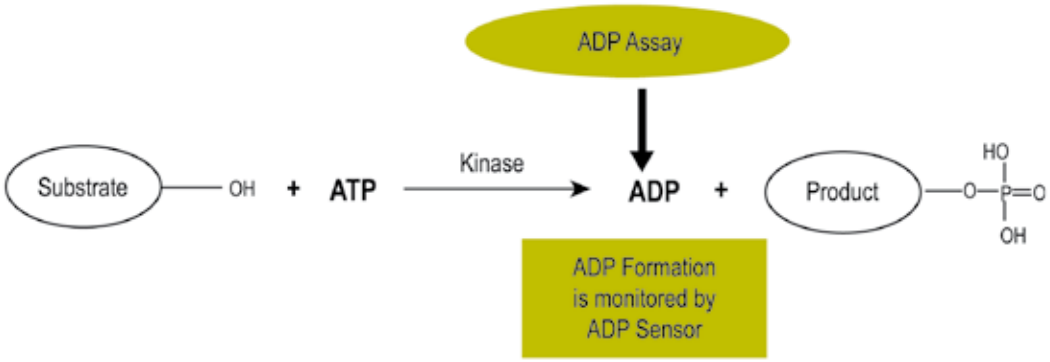



ATP dose response on 96-well white plate using 2 h incubation time (Z' factor = 0.6, Blue 30 min, red 1 h, and green 2 h). The integration time was 1 sec. The half life is more than 1.5 h.

| Description | P/N : | Qty |
|---|---|---|
| ATP Assay Kit, Bright Glow | **FN0640** | 96 assays |

## Universal Fluorimetric Kinase Assay Kit, Red Fluorescence

Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP deletion. For the kinase assay kits that are based on detection of phosphopeptides one has to spend time and efforts to identify an optimized peptide substrate while the ATP depletion method suffers various interferences due to the use of luciferase that are inhibited or activated by various biological compounds. The Universal Kinase Assay Kit is based on the monitoring of ADP formation, which is directly proportional to enzyme phoshphotransferase activity and is measured fluorimetricaly. This kit provides a fast, simple, and homogeneous assay for measure kinases activities. The characteristics of its high sensitivity (<0.2 uM ADP), broad ATP tolerance (1-300 uM), non-antibody based, non-radioactive and no-wash method to detect the amount of ADP produced as a result of enzyme activity make it an ideal kit for determining kinase Michaelis-Menten kinetics and for screening and identifying kinase inhibitors.



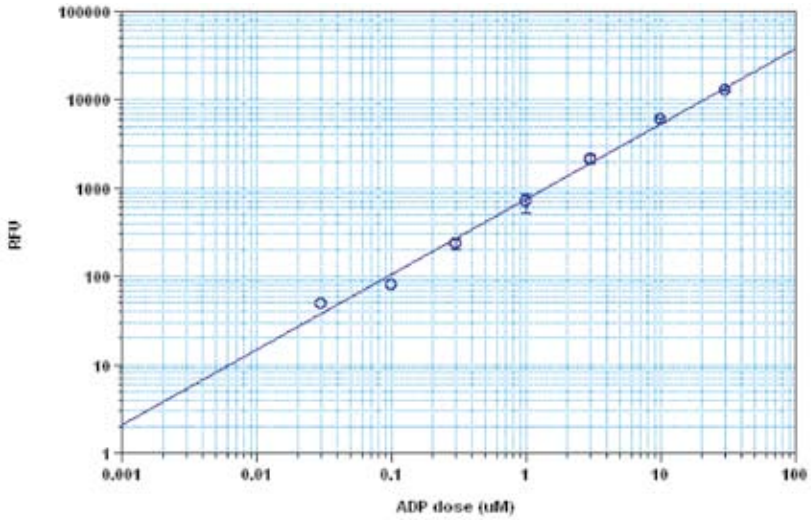

- Universal : Can be used for any kinases that used ATP as phosphate donor.
- Continuous : Easily adapted to automation with no mixing or separation protocols.
- Use of Native substrates : Substrates can be proteins, peptides or sugars.
- Non-Antibody-Based : No antibody is used in the kit.

| Description | P/N : | Qty |
|---|---|---|
| Universal Fluorimetric Kinase Assay Kit (540/590 nm) | **CL9170** | 250 assays |
| Contains : ADP sensor buffer, ADP Sensor, ADP standard, ADP Assay Buffer | | |



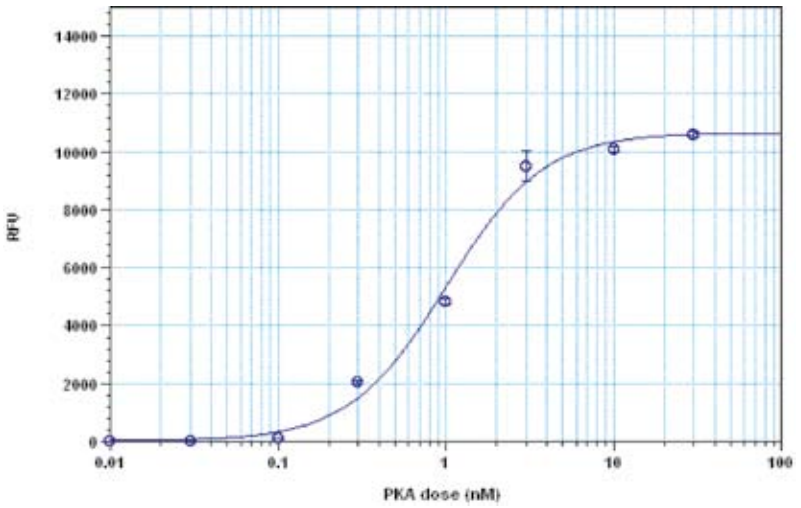
## ■ ADP Assay Kit, Red Fluorescence

Substrate : red fluorescent substrate
$\lambda_{ex./em.}$ : **571 / 585 nm**
Sensitivity : **0.2 µM ADP**
Large Range of ATP Tolerance : 1-300 µM.




ADP dose response on 384-well black plate with 15, 30 minutes and 1 hour incubation time (Z' factor =0.65).

ADP is involved in many biological reactions such as protein kinases. Our ADP assay kit can be used for assaying protein kinase reactions universally by monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This kit provides a fast, simple and homogeneous assay for measuring ADP formation or depletion. The assay is continuous, and can be easily adapted to automation. The kit is convenient, requiring minimal hands-on time. Protein kinases are of interest to researchers involved in drug discovery due to their broad relevance to diseases such as cancer and other proliferative diseases, inflammatory diseases, metabolic disorders and neurological diseases. Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP deletion. Our ADP Assay Kit is based on the monitoring of ADP formation, which is directly proportional to enzyme phoshphotransferase activity and is measured fluorimetricaly. This kit provides a fast, simple, and homogeneous assay for measure kinases activities.




Protein kinase A detection with incubation of the kinase in the presence of ATP and kemptide peptide substrate for 30 minutes.

| Description | P/N : | Qty |
|---|---|---|
| ADP Assay Kit, Red Fluorescence | **CI4171** | 100 assays |

## ■ Phosphate Assay Kit, Blue Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em}$ : **370 / 420 nm**

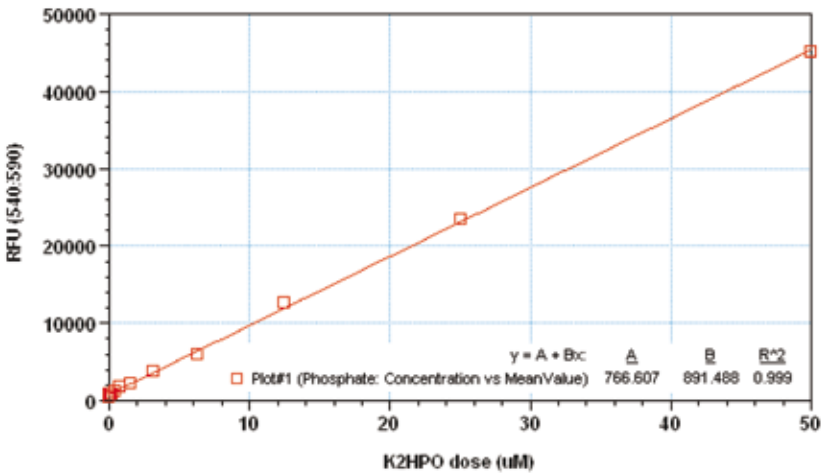
Phosphate is involved in many biological reactions. For example, phosphatases, ATPases and several other enzymes catalyze reactions in which inorganic phosphate (Pi) is released from a substrate. This Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme. The kit is formulated to give the simplest detection of Pi, neither coupling enzymes nor hazardous radioactive methods are involved. The measurement of Pi is based on our proprietary fluorescent sensor that has its fluorescence intensity proportionally dependent on phosphate concentration. Unlike other phosphate assays, this kit is easy to use. It is a mix and read format, and compatible with all the biological buffers.

| Description | P/N : | Qty |
|---|---|---|
| Phosphate Assay Kit, Blue Fluorescence | **JQ8120** | 1 kit |

## ■ Phosphate Assay Kit, Red Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em.}$ : **540 / 590 nm**
Sensitivity : **0.1 µM phosphate**

Cells utilize a wide variety of phosphate (Pi) and polyphosphate esters as enzyme substrates, second messengers, membrane structural components and vital energy reservoirs. Phosphate is involved in many biological processes. For example, phosphatases, ATPases and several other enzymes catalyze biochemical reactions in which inorganic phosphate is released from a phosphoester substrate. Detection of many phosphoester–metabolizing enzymes is difficult because suitable substrates are not available. It usually has been necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods. This Fluorimetric Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme using our red fluorescent phosphate sensor. The measurement of Pi is based on the change in the absorbance and fluorescence of our new phosphate sensor. Our kit provides all the essential reagents including phosphate sensor, phosphate standards and assay buffer. It can be used to measure the kinetics of phosphate release from phosphatases (such as GTPases and ATPases) by coupling the two enzymatic reactions. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required.




Phosphate dose response on 96-well black plate with 1 hr incubation time

| Description | P/N : | Qty |
|---|---|---|
| Phosphate Assay Kit, Red Fluorescence | **CI4161** | 100 assays |
| **Also available : colorimetric phosphate assays*** | | |
| Phosphate Assay, MG method<br>Original molybdate and malachyte green dye method. 600-660 nm reading. | **IS2790** | 1kit (600 assays) |
| Phosphate Assay, MG Plus method<br>Improved end-point stable signal (not prone to precipitation) | **CI4211** | 1kit (1000 assays) |

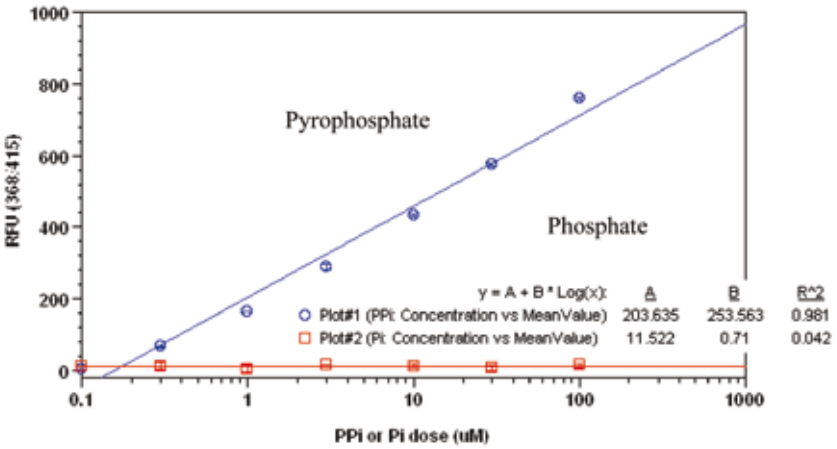
*The kit can also be used to estimate the phosphate content of proteins (phosphoserine or phosphothreonine post-translational modifications, after alkaline hydrolysis.




## ■ Pyrophosphate Assay Kit, Blue Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em.}$ : **370 / 420 nm**
Sensitivity : **0.3 µM** (30 pmoles) pyrophosphate

Pyrophosphate (PPi) are produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation by the enzyme adenylate cyclase and the enzymatic activation of fatty acids to form their coenzyme A esters. The Pyrophosphate Assay Kit provides the most robust spectrophotometric method for measuring pyrophosphate. This kit uses our proprietary fluorogenic pyrophosphate sensor that has its fluorescence intensity proportionally dependent upon the concentration of pyrophosphate. Our assay is much easier and more robust than the enzyme-coupling pyrophosphate methods that require at least two enzymes for their pyrophosphate detections. The kit provides all the essential components for assaying pyrophosphate.




| Description | P/N : | Qty |
|---|---|---|
| Pyrophosphate Assay Kit, Blue Fluorescence | **JQ8080** | 200 assays |

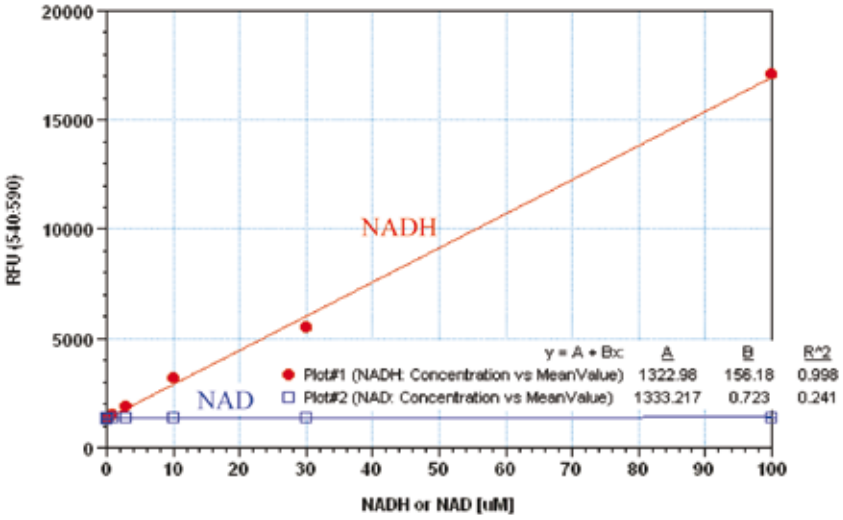
# NAD/NADH, NADP/NADPH Assay Kits

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+, and NAD+ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring of NADH or NADPH absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplate. Theses NAD/NADH & NADP/NADPH Assay Kits provide a convenient method for sensitive detection. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with 570 nm excitation 590 nm emission.

## ■ NADH Assay Kit, Red Fluorescence

Sensitivity : **10 nanomoles** of NADH in solution

The enzymes in the system specifically recognize NADH in an enzyme cycling reaction which significantly increases detection sensitivity.




NADPH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADP (the insert shows the lower detection limit).

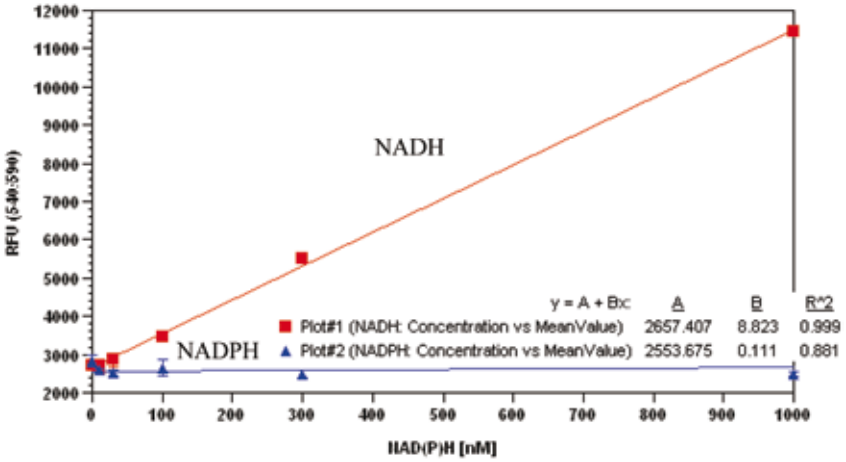
| Description | P/N : | Qty |
|---|---|---|
| Fluorimetric NADPH Assay Kit | **JQ7320** | 400 assays |

## ■ NAD/NADH Assay Kit, Red fluorescence

Sensitivity : **100 nM** (10 pmol/well) of NADH in solution

$\lambda_{exc./em.}$: **570/590 nm**

The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity.




NADH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADPH.

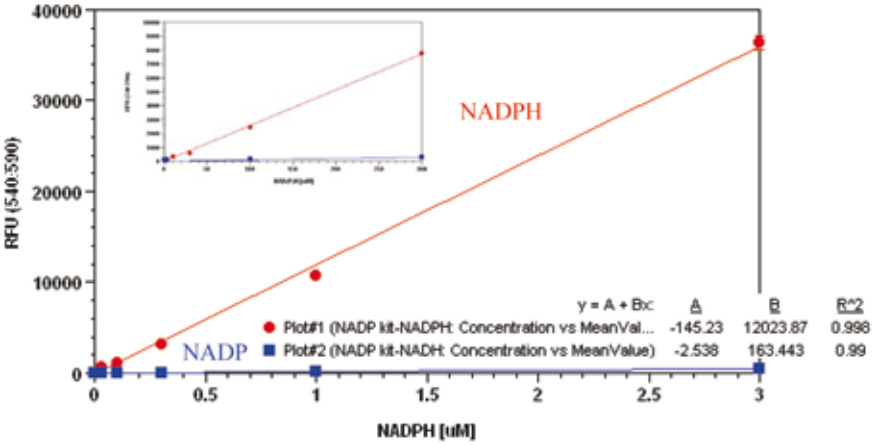
| Description | P/N : | Qty |
|---|---|---|
| NAD/NADH Assay Kit, Red fluorescence | **JQ7280** | 400 assays |



## NADPH Assay Kit, Red Fluorescence

Sensitivity : **30 nM** (0,3 nmol/well) of NADPH in solution

The enzymes in the system specifically recognize NADPH in an enzyme cycling reaction. The enzyme cycling reaction significantly increases detection sensitivity.



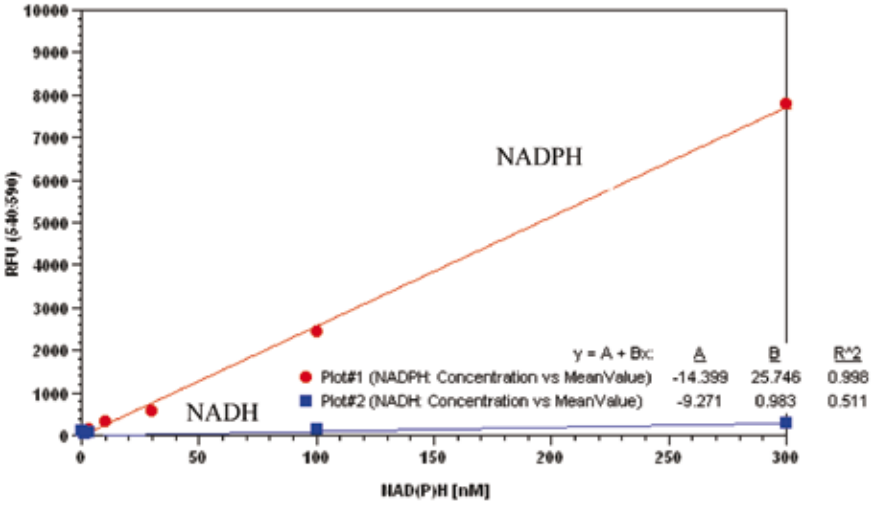

NADPH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADP (the insert shows the lower detection limit).

| Description | P/N : | Qty |
|---|---|---|
| Fluorimetric NADPH Assay Kit, Red fluorescence | **JQ7330** | 400 assays |

## NADP/NADPH Assay Kit, Red fluorescence

Sensitivity : **10 nM** (1 pmol/well) of NADPH in solution

This NADP/NADPH Assay Kit provides a convenient method for sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity.




NADPH dose response on 96-well black plate was measured with NADP/NADPH Assay Kit with 30 min incubation time (n=3) while there is no response from NADH.

| Description | P/N : | Qty |
|---|---|---|
| NADP/NADPH Assay Kit, Red fluorescence | **JQ7300** | 400 assays |