

Cell Biology - Assays Kits

Cell counting, viability and proliferation

Technical tip

MicroPlate readers

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



instruments.

*NightOWL LB981



*Mithras LB940 MultiMode Reader



Cell counting, viability and 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.

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 8 exclusion	Fluorimetric	++	++	+++	Cheap, Scalable, Non toxic. Do not state on viability More rapid than MTT/XTT ; unfixed or fixed samples
MTT	same, more soluble	Colorimetric	-	++	+++	Popular method. Sensitive, Scalable,
XTT	conversion in soluble orange	Colorimetric		++	+++	Non toxic Increased solubility and performance from MTT to XTT and WST
WST	formazan					
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, immunocytochemistry, flow cytometry, and in vivo cell tracing. Do not work for bacteria May alter some cell functions
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	conversion in colored product		-	-/+	+++	Recommended for cytotoxicity assays Serum interference
Luciferin / ATP measure		Luminescence	-	+	+++	Pros : sensitivity / linearity Cons : signal depends on each cell line, on temperature
DNA relase / -3H Thy -BRDU- ...						Recommended for cytotoxicity assays Cons :hazardous (radioelements)
⁵¹ Cr release Eu ³⁺					+++	Recommended for cytotoxicity assays Cons :hazardous (radioelements)
Propidium Iodide, 7-Membrane permeability AAD		Fluorimetric	+++	-	-	Used in combinaison of green fluorescence dye like Annexin V-FP488 to discriminate dead cells from alive cells

Technical tip

Review of Counting / Proliferation / viability Cell Assays

Since the early 1900s, researchers have used the vital stain **trypan blue** to differentiate live cells from dead or dying cells. Trypan blue was found to be an ideal stain for this purpose because it easily diffuses across cell membrane of dead or dying cells, but cannot cross membranes of live cells (Evans). Cell counting performed visually by microscopy, but this is time consuming and not convenient for numerous samples, nor possible if cells are cultured on support that disaggregate (ceramics, HA). Counting is now also performed in automates.

Tritiated thymidine method, based on the incorporation of H_3 -thymidine into DNA during cell growth, was also very useful. However, this method requires hazardous materials that causes expensive and safety drawbacks for operating and disposal.

Besides their specific limitations, these methods cannot process large numbers of samples.

The use of tetrazolium salts, including MTT and XTT **formazan dyes**, to assay cell proliferation, cell viability, and/or cytotoxicity is now a widespread, established practice. The procedure is safe, allows rapid determination in microplates, and give reproducible and sensitive results. The tetrazolium salt are converted in metabolically active cells by cytoplasmic enzymes, generating a staining. The reaction is attributed mainly to mitochondrial enzymes and electron carriers, but a number of other non-mitochondrial enzymes have been implicated. These dyes are however toxic, with safety and disposal concerns for the users, and limitations for long-term studies.

Though these methods are still commonly used as a measure of cell proliferation, limitations prompted the development of a number of new technologies to monitor cell proliferation, viability, and more specific parameters, and/or to improve sensitivity, minimize interferences (longer studies, reluctant cells,...), increase sensitivity or ease use and scalability. Most of these technologies are fluorescence-based, doing away with the need for radioactivity, some others are dedicated to colorimetry or immunocytochemistry. For example,

- ◆ New membrane integrity probes have replaced Trypan, for IHC (**Hoechst**), for flow cytometry (**Calcein**), and for apoptosis (**AnnexinV**, **Propidium Iodide**).
- ◆ A remarkable cell proliferation assay is now **UptiBlue** which replaces advantageously MTT/XTT, measuring cytoplasmic membrane redox potential, one major energy state of cells : it is more sensitive, non-toxic to cells nor technicians. Applications range from scalable cell counting to HTS screening proliferation assays. See page E150.
- ◆ Specific probes allow more specific cell viability measurement, based on cytoplasmic and mitochondrial redox potential (**JC-1**), enzyme reduction (**WST**), and DNA detection (anti **BrDU**). Even more specific probes allow to get information useful for cell physiologic response in immunology, cancerology, apoptosis...

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Cell Biology - Assays Kits

Cell counting, viability and proliferation

Vital stains based Dead Cell staining (Trypan, Hoechst, EB/DAPI)

Hereafter are presented the standard Trypan blue and minor-groove DNA binder stains (Hoechst, PI and DAPI) essentially used for counting dead cells.

Trypan Blue based Cell stain

3,3'-[[3,3'-Dimethyl-(1,1'-biphenyl)]-4,4'-diyl]-bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid), tetrasodium salt

$C_{34}H_{24}N_6O_{14}S_4Na_4$, MW : 960.81

Soluble in water

EC (606 nm) > 69 000

Trypan Blue is commonly used for dead cell staining, in so called the dye exclusion test. Trypan Blue does not stain viable cells. Therefore, dead Trypan Blue-stained cells are easily recognized by microscopy and can be counted using a hemacytometer. Erythrosin B, negrosine, eosin Y, Acridine Orange and Ethidium Bromide are also utilized for this purpose. Though it is hard to detect cells in early to middle stages of apoptosis, Trypan Blue staining is a very simple and widely used method to visualize dead cells.

Description	Cat.#	Qty
Trypan stain	T33190	5 g

Hoechst Cell Proliferation Assays

For the determination of cell number and proliferation.

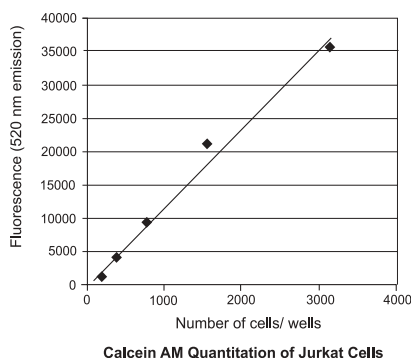
This is a rapid quantitative method for measuring the absolute number of cells for both proliferating and non-proliferating cells in suspension or adherent cell culture. The assay does not require radiation or the use of toxic reagents, and is time sensitive for those researchers screening the effects of apoptosis-modulators, cytotoxic agents and regulators of cell division. Select your Hoechst CPA kit based on whether a vital dye is required (Hoechst CPA1 kit) or if fixed cells will be assayed (Hoechst CPA2 kit).

Features :

- ◆ Ideal for both suspension and adherent cells.
- ◆ Non-radioactive and non-toxic format.
- ◆ Rapid and time sensitive method, scalable.
- ◆ Applicable to unfixed or fixed samples.

Applications :

- ◆ Cell counting
- ◆ Cell proliferation



Description	Cat.#	Qty
Hoechst CPA1 TACS™ Hoechst Cell Proliferation Assay	Q70130	1 kit 2500 tests
Contains : CPA Dye 1(Hoechst 33342)	Q70110	1.25 ml
CPA Dilution Buffer	Q70120	500 ml
Hoechst CPA2 TACS™ Hoechst Cell Proliferation Assay	Q70150	1 kit 2500 tests
Contains : CPA Dye 2 (Hoechst 33258)	Q70140	1.25 ml
CPA Dilution Buffer	Q70120	500 ml

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EB/PI/DAPI fluorescent stains

Description	Cat.#	Qty
Ethidium Bromide (EB, BET) stain	FP-06022A	5 x 1 g
	FP-32790A	5 ml at 0.625 mg/ml (dropper)

EB does not cross viable cell membranes. However, it passes through the disrupted membranes of dead cells to stain nucleic DNA. The excitation and emission wavelengths of EB-DNA complex are 518 nm and 605 nm, respectively. EB is a potent mutagen.

EB-DNA complex
 $\lambda_{ex}/\lambda_{em}$: 518/605 nm

Description	Cat.#	Qty
Propidium Iodide (PI) stains (dead cell staining)	31238B	100 mg
	FP-36774A	10 ml at 1mg/ml in water

Though PI does not cross viable cell membranes, it passes through disturbed cell membranes and stains the nucleus. PI is often used in combination with green fluorescent compound, such as Calcein-AM, FDA or Annexin V-FluoProbes® 488, for simultaneous staining of viable and dead cells. The excitation and emission wavelengths of PI-DNA complex are 535 nm and 615 nm, respectively.

PI-DNA complex
 $\lambda_{ex}/\lambda_{em}$: 535/615 nm

Description	Cat.#	Qty
DAPI stain (dead cell staining)	FP-371867	10 mg
	AP4460	50 tests
	AP4461	2 x 625 tests

Though DAPI does not cross viable cell membranes, it passes through disturbed cell membranes to stain the nucleus. DAPI is used for the detection of mitochondrial DNA in yeast, chloroplast DNA, virus DNA, mycoplasma DNA and chromosomal DNA. The excitation and emission wavelengths of DAPI-DNA complex are 360 nm and 460 nm, respectively. DAPI is carcinogenic.

DAPI-DNA complex
 $\lambda_{ex}/\lambda_{em}$: 360/460 nm

See more information and other vital stains in section "DNA probes" (page E119).

Cell Biology - Assays Kits

Cell counting, viability and proliferation

Redox probes based Cell assays



UptiBlue Viable cell stain

UptiBlue is a unique assay for cell counting, applied to proliferation assays as well cytotoxicity screening.

Features	Benefits
Sensitive	More sensitive than MTT/XTT*
Works in both Fluorescent and spectrophotometer	Allows choice of detection method : either with a spectrophotometer or a spectrofluorimeter*
Compatible with various samples	Works also for bacteria and fungi Works on suspension or attached cell lines* No interference from the presence of 10% fetal bovine serum, nor from phenol red in the growth medium.
Water soluble.	No solubilization nor cell extraction required !*
Non-toxic to cells	Allows continuous cell growth monitoring, kinetic studies, incubation time of days*
Non-toxic to technician	Safe, disposable, less regulation*
Easy-to-use	Time saving, easily adaptable to automation, as microplate readers * Fewer steps than MTT technique * No required centrifugation! * Stable (12 months at RT, 20 months at 2-8°C, or indefinitely at -70°C)

*advantages over MTT/XTT are noted with an asterix

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.

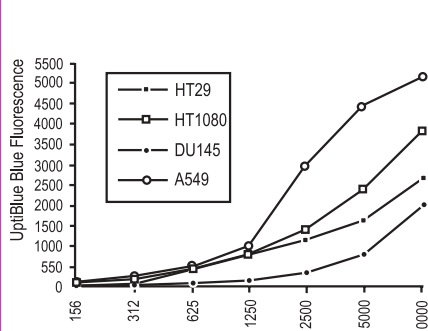
UptiBlue shows excellent correlation to formazan and tritiated thymidine techniques, while being much easier and safer to use. It especially replace advantageously MTT/XTT in many applications, from cell counting to proliferation assay and cytotoxicity testing. Furthermore it allows longer studies.

Description	Cat.#	Qty
UptiBlue Viable cell counting reagent	UP669412	25 ml
	UP669413	100 ml

Technical tip

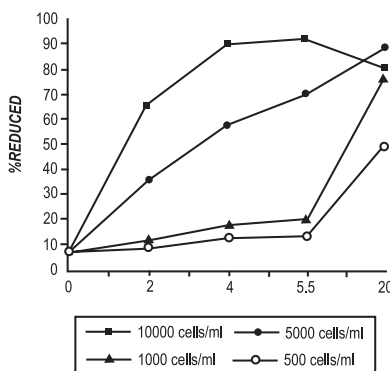
Applications

Cell proliferation assay



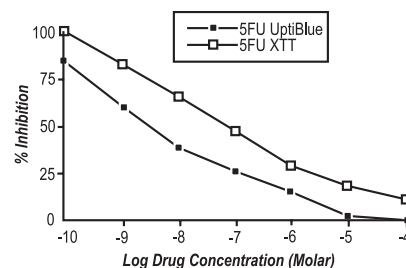
Detection of cell Growth of 4 Cell Lines using UptiBlue

Kinetic / long term assays



Kinetic reduction curve with UptiBlue with plating density from 500 to 10,000 cells A549/ml.

Cytotoxicity assay



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

Determination of Doxorubicin LD₅₀ using UptiBlue and XTT

See also resorufin probe.

Formazan based cell assays

The use of tetrazolium salts is a widespread and established practice to assay cell proliferation, cell viability, and/or cytotoxicity. Here are described 3 kits with the conventional MTT dye, the non-toxic XTT dye, and the innovative superior WST-8 dye.

CCK Cell Counting kit

- ◆ Colorimetric microplate assay
- ◆ Ready-to-use one-bottle solution
- ◆ No radioisotope or organic solvent required
- ◆ No toxicity to cells
- ◆ No harvesting, washing or solubilization step required
- ◆ More sensitive than MTT, XTT, MTS or WST-1

The cell proliferation assay procedure is very simple. No pre-mixing of components is required. Ten μ l of the kit solution is added to each well of the plate on which is inoculated 100 μ l of the cell suspension. After the plate is incubated for 1-4 hours in the incubator, the absorbance is measured using a microplate reader. The wavelength range for the measurement of the absorbance is between 450 nm and 490 nm. So the researcher is able to choose a popular filter, between 450 nm and 490 nm. The kit solution is stable for over 1 year at -20 °C and over 3 months at 4 °C with protection from light.

The standard procedure needs 5 000-10 000 cells for optimal measurements. For adhesive cells, at least 1 000 cells are necessary per well. For leukocytes, at least 2 500 cells are necessary per well. The recommended maximum number of cells per well for the 96-well plate is 25 000.

Principle :

CCK-8 consists of WST-8 (5 mM) and 1-methoxy PMS (0.2 mM) as an electron mediator. PMS, receives electrons from viable cells and transfers the electrons to WST-8 in the culture medium. The amount of the yellow colored formazan dye generated in tissue culture medium is directly proportional to the number of viable cells.

Furthermore, the cell proliferation assay data using CCK-8 correlates with that using the 3H-thymidine incorporate assay.

Compared to MTT/XTT/MST salts methods,

- ◆ The sensitivity using CCK-8 is higher
- ◆ The procedure is considerably simplified.

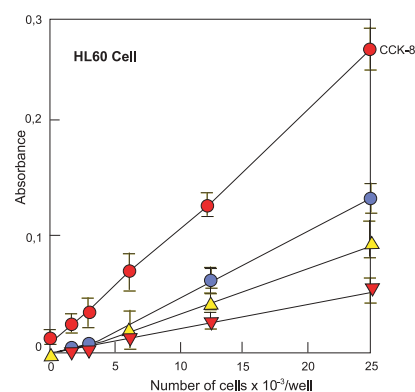
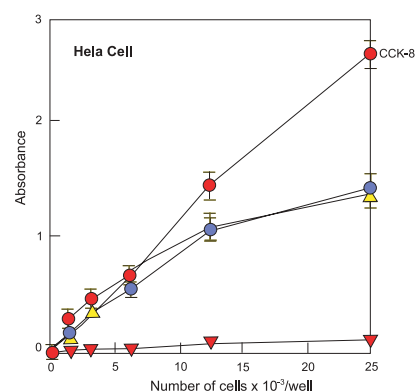
The kit performs advantageously :

- ◆ In clinical analysis, the sensitivity of the detection of analytes using WST is equal or greater than conventional tetrazolium salts such as INT, MTT or NBT.
- ◆ In cytotoxicity assay, WST8 minimizes the process to determine living cell number. Therefore, the cytotoxicity assay using WST is suitable for the first screening of the determination of cytotoxicity of chemicals, and contributes to reduce the consumption of experimental animals.

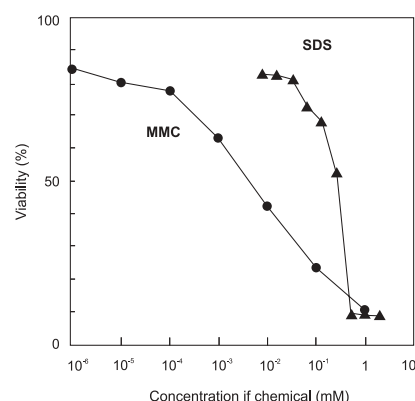
Description	Cat.#	Qty
CCK Cell Proliferation Assay	899650	1000 tests
	899651	3000 tests

Toxicological test of chemicals by using CCK-8

Cell line : HeLa
Culture medium : MEM, 10% FCS, L-glutamine
Chemical : mitomycin-C (MMC)
dodecylsulfate, sodium salt
Staining : 37°C, 5% CO₂, 2 hours
Detection : 450 nm, reference 650 nm

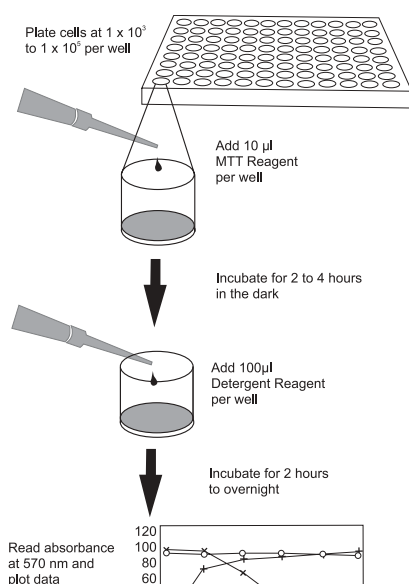


Detection Methods
CCK-8 : 450 nm, reference : 650 nm
XTT : 450 nm, reference : 650 nm
MTS : 490 nm, reference : 650 nm
MTT : 570 nm, reference : 650 nm



Cell Biology - Assays Kits

Cell counting, viability and proliferation



TACS™ MTT - Cell Proliferation Assay

A sensitive kit for the measurement of cell proliferation based upon the reduction of MTT. The reduction of MTT in an insoluble colored formazan is primarily due to glycolytic activity within the cell and is dependent upon the presence of NADH and NADPH (thus associated to mitochondrial metabolic activity).

In actively proliferating cells, increase in MTT conversion is spectrophotometrically quantified. Comparison of this value to an untreated control provides a relative increase in cellular proliferative activity caused i.e. by trophic factors, growth inhibitors, or inducers and inhibitors of apoptosis, which may be quantified. Conversely, in cells that are undergoing apoptosis, MTT reduction decreases, reflecting the loss of cell viability.

Features :

- ◆ Convenient. Stabilized formulation is stored in your refrigerator and does not require thawing before use.
- ◆ Non-isotopic. Assay for cell proliferation, cytotoxicity, and viability does not require isotopic reagents.
- ◆ Fast. High throughput microplate format.
- ◆ Flexible. The reaction product can be visualized directly by microscopy to evaluate cell to cell reactivity, or solubilized and evaluated by microplate reading.
- ◆ Safe. Reaction product is solubilized using a non-organic solvent.

Applications :

- ◆ Cell proliferation assays
- ◆ Cytotoxicity analysis
- ◆ Apoptosis screening

Description

Description	Cat.#	Qty
MTT Cell Proliferation Assay	45547A	1000 Tests
MTT Cell Proliferation Assay	455470	2500 Tests
MTT Cell Proliferation Assay	455471	5000 Tests
contains : MTT reagent	Q70090	25 ml
Detergent reagent	Q70100	250 ml

also available :

MTT powder	FP-65939	1 g
3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium, $C_{18}H_{16}BrN_5S$; MW : 414.33		

TACS™ XTT Cell Proliferation assay

XTT, a yellow tetrazolium salt, is cleaved in a soluble orange formazan dye, which can be measured by absorbance at 490 (or 450) nm in a microplate reader. This kit includes both XTT and an electron coupling reagent for efficient reduction in a convenient and simple assay. The assay is safer than triated thymidine method, faster and more convenient than MTT method. Low cell number can be used, and it is scalable.

Features :

- ◆ Sensitive.
- ◆ No radioactivity.
- ◆ Rapid (no solubilization step as in a MTT assay).
- ◆ Ideal for high throughput assays (no washing or other steps that can cause cell loss and variability).

Applications :

- ◆ Cell proliferation assays
- ◆ Cell viability assays
- ◆ Cytotoxicity analysis

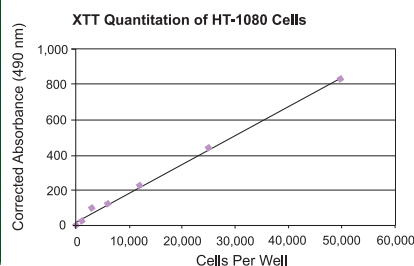
Description

Description	Cat.#	Qty
TACS™ XTT Cell Proliferation assay	FX8730	250 tests
contains : XTT reagent (M)	FX8710	5 x 25 ml
XTT activator (M)	FX8720	5 x 500 µl

also available :

XTT powder	FP-40936A	100 mg
2,3-Bis(2-methoxy-4-nitro-5- sulfophenyl)-2H-tetrazolium-5-carboxanilide; $C_{22}H_{17}N_7NaO_{13}S_2$; MW : 674.53 ; Irritant		

E.152



Quantitation of HT-1080 cells using XTT. HT-1080 cells were serially diluted in DMEM and incubated for two hours with XTT. Absorbance values were obtained at 490 nm in a 96 well plate reader.

ATP Cell Viability Assay Kit

Features :

- ◆ High Sensitivity and Extended Linearity
- ◆ Simple : a single-step homogenous assay
- ◆ Robust & Amenable to HTS

This assay use a thermostable firefly luciferase system. It is ideally suitable for sensitive quantification of ATP in various applications such as cell proliferation, ATP detection in biological samples or monitoring of ATP dependent enzyme assays (kinases). The assay are available in two different kit formats :

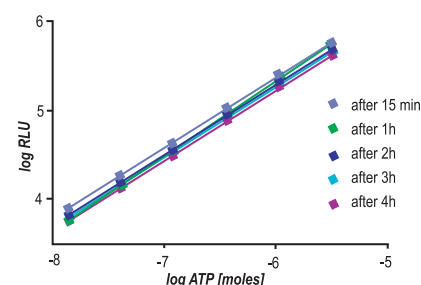
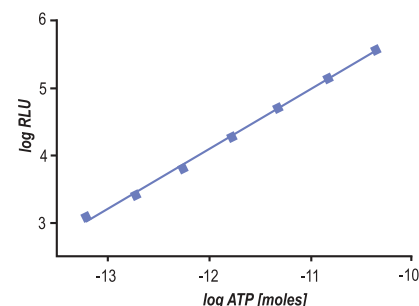
- ◆ The ATP **high sensitivity** determination kit is optimized to detect very small amounts of ATP in various samples. Even 0.1pmol of ATP can accurately be determined.
- ◆ The ATP **time-stable** determination kit is optimized when signal stability is sought, as in High Throughput Screenings, and for 10 nM to 10μM ATP concentrations. The luminescence signal is stable for at least 4 hours, with excellent Z'-factor values.

10 ml of the assay mix is sufficient to perform at least 200 analyses depending on assay volume.

Applications :

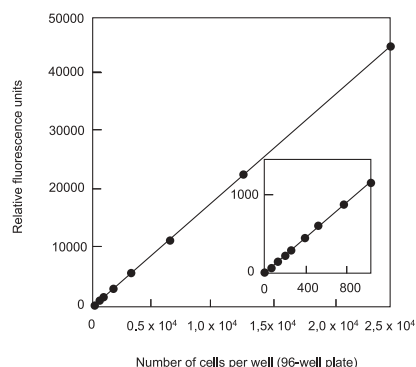
- ◆ Cell viability
- ◆ Kinase assays

Description	Cat.#	Qty
ATP Determination Kit, high sensitivity assay	S2841	10 ml
	S2842	10 x 10 ml
	S2841	100 ml
ATP Determination Kit, time-stable assay	BU1200	10 ml
	BU1201	10 x 10 ml
	BU1202	100 ml



Cell Biology - Assays Kits

Cell counting, viability and proliferation



Typical calibration curve :

Technical tip

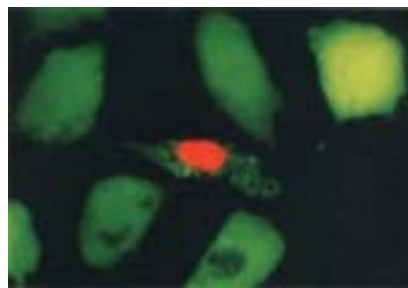
Calcein cell assays limitations

If longer observation period is necessary, please try CFDA-SE staining. As Calcein-AM cannot pass through bacterial cell walls, you may alternatively try UptiBlue. #UP66941 (page E150) Calcein binds calcium ion in the cell, so the reduction of the free calcium ion may effect cell functions.

Related products :

Individual dyes of this kit and other cell tracing dyes are presented in other sections (J) :

Description	Cat.#
CFDA-SE (CFSE) (see section "Cell tracers") page E96	FP-52493A
BCECF-AM (see section "pH indicators") page E65	FP-45440A



Please see Table B1 for comparison with other Viability/ Cytotoxicity Assay Kits.

Calcein based cell viability assays

Cell Counting kit - Fluorescent, based on Calcein-AM

Cell Counting Kit-F is utilized for the fluorometric determination of living cell numbers. The amount of a fluorescent dye, calcein, hydrolyzed by esterases in cells is directly proportional to the number of viable cells in culture media. The 96-well microplate CCK-F assay has a detection range of less than 50 cells to more than 25 000 cells 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. Excitation and emission occur at 485 (460-490) nm and 535 (510-540) nm, respectively. An incubation of 10 to 30 minutes gives sufficient fluorescence intensity for the cell viability determination.

Description	Cat.#	Qty
Cell Counting kit - Fluorescent, Based On Calcein-AM	876981	500 tests
	876982	2 x 500 tests

Cell Counting kit - Double Staining Kit (Calcein AM + PI)

Cellstain-Double Staining Kit is used for simultaneous fluorescence staining of viable and dead cells. This kit contains Calcein-AM and Propidium Iodide (PI) solutions, which stain viable and dead cells, respectively. Since both calcein and PI-DNA can be excited with 490 nm, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope or flow cytometry. With 545 nm excitation, only dead cells can be observed. Since optimal staining conditions differ from cell line to cell line, a suitable concentration of PI and Calcein-AM should be individually determined. As PI is suspected to be highly carcinogenic, careful handling is required.

Description	Cat.#	Qty
Cell Counting kit - Double Staining Kit (Calcein AM + PI)	486301	1 kit
Contains : 1 vial of PI, 4 vials of Calcein AM		

Viability/Cytotoxicity Staining Kit for Live & Dead Cells Calceins (AM + EB)

Viability/cytotoxicity Staining Kit for Live & Dead Cells provides a two-color fluorescent staining of live (green) and dead cells (red) using two probes. Calcein AM stains live cells green while EthD-III stains dead cells red. These probes measure two recognized parameters of cell viability — intracellular esterase activity and plasma membrane integrity. The kit is suitable for use with fluorescence microscopes, fluorescence multi-well plate scanners and flow cytometers. The assay principle apply to most eukaryotic cell types, including adherent cells and certain tissues, but not to bacteria or yeast. This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, ⁵¹Cr release and similar methods for determining cell viability and cytotoxicity. EthD-III (#BP934) is a superior alternative to EthD-I offered by our competitors. EthD-III has higher DNA binding affinity and thus brighter fluorescence (70% brighter than Ethidium homodimer I).

Features :

- ◆ 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

Description	Cat.#	Qty
Viability/Cytotoxicity Staining Kit for Live & Dead Cells	BF4710	300 assays

E.154

LDH based cell proliferation assays

Lactate dehydrogenase (LDH) is ubiquitously present in mammalian cells. The measurement of cytoplasmic LDH activity is a well-accepted assay to quantify cell numbers and indicate cell viability. Upon cell death, LDH is released into the surrounding medium.

DHL™ Cell Viability and Proliferation Assay Kit "Fluorimetric"

The DHL™ Cell Viability and Proliferation Assay Kit # HT6340 provides researchers with a convenient one-step assay to measure LDH activity in living cells. Using this kit, one can continuously monitor cell proliferation over time (Figure 1). Resazurin is used in this kit as a sensitive indicator. It is converted to the strongly fluorescent resorufin by cytoplasmic LDH in living cells (diagram 1). The kit can detect as few as 48 cells, to 100 000+. It is suitable for high throughput screening of cell proliferation or cytotoxicity effect of a variety of compounds.

D. W. De Jong and W. G. Woodlief, *Biochim.Biophys.Acta* 484, 249-259 (1977).
C. Korzeniewski and D. M. Callewaert, *J.Immunol.Methods* 64, 313-320 (1983).

DHL™ Express Cell Counting Kit

Complimentary to kit #HT6340, The DHL™ Express Cell Counting Kit # BP7070 provides researchers with a convenient 15-minute assay to quantify total cell number in a culture, including living and dead cells, by measuring total LDH activity in both cytoplasm and culture medium. The kit uses resazurin, a sensitive indicator, which can be converted to the strongly fluorescent resorufin (excitation/emission : 530-560 nm /590 nm) by LDH in a coupled enzymatic reaction (diagram 1). The kit can detect as few as 97 cells with a linear range of up to 2.5×10^4 cells ($r^2 : 0.95$). It is suitable for high throughput screening of cell proliferation or cytotoxicity effect of a variety of compounds.

Larson EM, et al. (1997). A new, simple, nonradioactive, nontoxic in vitro assay to monitor corneal endothelial cell viability. *Invest Ophthalmol Vis Sci* 38, 1929-33.

Description	Cat.#	Qty
DHL™ Cell Viability and Proliferation Assay Kit *Fluorimetric*	HT6340	1000 tests*
* The kit contains 20 ml assays solution to perform 1000 Assays (96-well) or 2000 Assays (384-well)		
DHL™ Express Cell Counting Kit *Fluorimetric*	BP7070	500 tests
The kit contains : Assay mixture, Assay buffer, Lysis solution, Stop solution to perform 500 Assays (96-well) or 1000 Assays (384-well)		

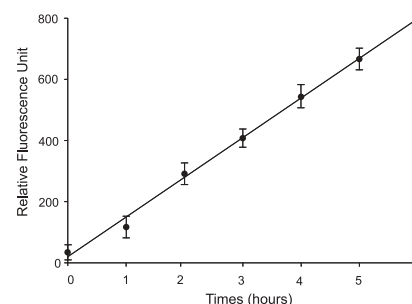


Figure 1. Continuous measurement of cell proliferation by monitoring the change in fluorescence signals. 1×10^4 Jurkat cells were seeded into a 96-well plate. 20 μ L of assay solution was added and incubated in a 37°C incubator. The fluorescence signal was monitored at $\lambda_{ex}/\lambda_{em}$: $530 \pm 30/590 \pm 30$ nm for up to 5 hours. (mean \pm S.D., n=four independent samples).

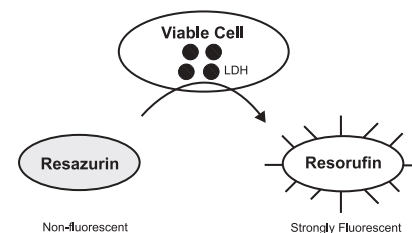


Diagram 1. The conversion of resazurin to the strongly fluorescent resorufin by dehydrogenases (e.g. LDH) in living cells.

See also LDH Cytotoxic assays (#HT0271, T)