Measuring Cell Viability / Cytotoxicity

Introduction

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. Fig. 1 indicates various reagents used for cell viability detection. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Many have established methods such as Colony Formation method, Crystal Violet method, Tritium-Labeled Thymidine Uptake method, MTT, and WST methods, which are used for counting the number of live cells.

Trypan Blue is a widely used assay for staining dead cells. In this method, cell viability must be determined by counting the unstained cells with a microscope or other instruments. However, Trypan Blue staining cannot be used to distinguish between the healthy cells and the cells that are alive but losing cell functions. In the Colony Formation method, the number of cell colonies are counted using a microscope as a cell viability indicator. In the Tritium-Labeled Thymidine Uptake method, [^{3}H]-thymidine is involved in the cell nucleus due to the cell growth, and the amount of the tritium in the nucleus is then measured using a scintillation counter. Though the Tritium-labeled thymidine uptake assay is sensitive to determine the influence on the DNA polymerization activity, it requires radioisotope which causes various concerns.

The ^{51}Cr method is highly sensitive, and is commonly used to determine low levels of cytotoxicity. However, the use of ^{51}Cr also causes problems in handling, storage, and disposal of the material.
Cellular enzymes such as lactate dehydrogenase, adenylate kinase, and glucose-6-phosphate dehydrogenase are also used as cell death markers, and there are several products available on the market. However, adenylate kinase and glucose-6-phosphate are not stable and only lactate dehydrogenase does not lose its activity during cell death assays. Therefore, cell death assays based on lactate dehydrogenase (LDH) activity are more reliable than other enzyme-based cell death assays.

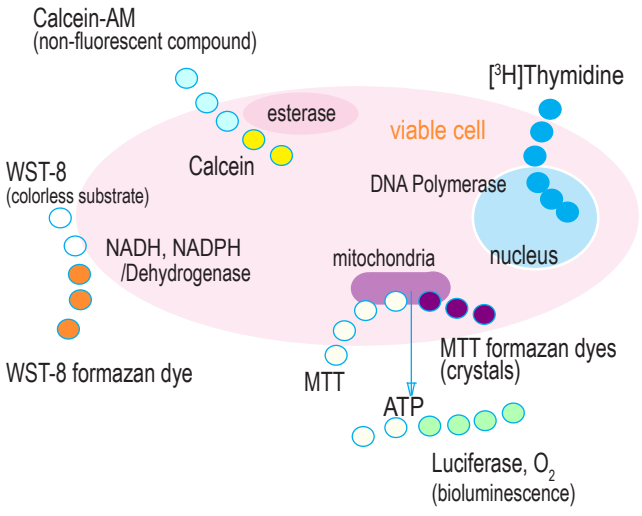

Fig. 1 Reagents for cell viability detection

Enzyme-based methods using MTT and WST rely on a reductive coloring reagent and dehydrogenase in a viable cell to determine cell viability with a colorimetric method. This method is far superior to the previously mentioned methods because it is easy-to-use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. Therefore, this method is suitable for those who are just beginning such experiments. Among the enzyme-based assays, the MTT assay is the best known method for determining mitochondrial dehydrogenase activities in the living cells. In the method, MTT is reduced to a purple formazan by NADH. However, MTT formazan is insoluble in water, and it forms purple needle-shaped crystals in the cells. Therefore prior to measuring the absorbance, an organic solvent is required to solubilize the crystals. Additionally, the cytotoxicity of MTT formazan makes it difficult to remove cell culture media from the plate wells due to floating cells with MTT formazan needles, giving significant well-to-well error.

Dojindo developed highly water-soluble tetrazolium salts called WSTs. WSTs produce water-soluble formazans and are suitable for cell proliferation and cytotoxicity assays. WSTs receives two electrons from viable cells to generate a yellow, orange, or purple formazan dye. WST-8, a highly stable WST, is utilized in Cell Counting Kit-8 (CCK-8). The electron mediator used in this kit, 1-Methoxy PMS, is also highly stable. Therefore, CCK-8 is stable for at least 6 months at the room temperature and for one year at 0-5 °C. Since WST-8, WST-8 formazan, and 1-Methoxy PMS have no cytotoxicity in the cell culture media, additional experiments may be carried out using the same cells from the previous assay.

Dehydrogenase-based assays reflect cell conditions with more sensitivity than the other assays because they depend on several elements including dehydrogenase, NAD(H), NADP(H), and mitochondrial activity. The major difference between CCK-8 and the MTT assay, other than MTT's toxicity, is the enzymes involved. The CCK-8 assay involves most of the dehydrogenase in a cell. On the other hand, MTT only involves mitochondrial dehydrogenase. Therefore, the MTT assay depends on mitochondrial activity, not the cell itself. Additionally, CCK-8 is far more sensitive than the MTT assay. Since WST-8 formazan is water soluble, it does not form crystals like MTT. Therefore, after 1-4 hours of incubation with the CCK-8 solution, measurement of O.D. at 450 nm gives the number of viable cells. No extra steps are required.

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Measuring Cell Viability/Cytotoxicity: Cell Counting Kit-8

Product Description

Cell Counting Kit-8 is a colorimetric assay for the determination of viable cell numbers and can be used for cell proliferation assays as well as cytotoxicity assays. Cell Counting Kit-8 uses a tetrazolium salt, WST-8, which produces the water soluble WST-8 formazan. Since this orange colored formazan does not require dissolving, no solubilizing process is required. Results are obtained after 3 simple steps: 1) add, 2) incubate, and 3) read. This kit is applicable for 96-well microplate assays and can also be applied to High-Throughput Screening such as a 384-well microplate. WST-8 is not cell permeable, which results in low cytotoxicity. Therefore after the assay it is possible to continue further experiments using the same cells.

Applications: cell counting, cell proliferation experiments, cytotoxicity tests, drug sensitivity tests

Product Information

Cell Counting Kit-8

Product code	Unit	Components
CK04-01	100 tests	1 ml bottle x 1
CK04-05	500 tests	5 ml bottle x 1
CK04-11	1,000 tests	5 ml bottle x 2
CK04-13	3,000 tests	5 ml bottle x 6
CK04-20	10,000 tests	100 ml bottle x 1

One test corresponds to one well of the 96-well plate.

Required Materials

Devices, Tools

- Microplate Reader with a 450 - 490 nm filter
- 96 well microplate, sterilized clear plate for cell assay
- Multi-channel pipette (8 or 12 channel: 10-100 μl)
- Pipette tips for 10-100 μl
- CO_2 incubator
- Clean bench
- Hematocytometer or cell counter
- Centrifuge and rotor for a 15 ml centrifuge tube

Reagents

- Cell Counting Kit -8 [product code: CK04]
- Cell culture media
- Material to be tested
- PBS or other buffers for the preparation of material solutions if cell culture medium cannot be used.

Preparation

Cell Counting Kit-8

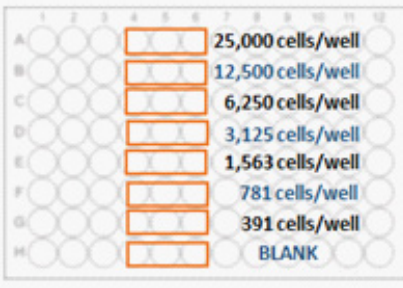
- Ready-to-use one solution
- Stable for 12 months when stored at 4 °C

If you use Cell Counting Kit-8 frequently, store in a refrigerator. The solution is stable for one year at 4 °C. The solution is also stable at room temperature for 6 months.

If you plan not to use the Cell Counting Kit-8 for more than a year, aliquot the Cell Counting Kit-8 solution and store in a freezer at -20 °C to avoid repeated freeze and thaw.

Optimization of Assay Condition

When using Cell Counting Kit-8 for proliferation and cytotoxicity assays, it is necessary to have a proportional relationship between absorption and viable cell numbers. It is desirable to start with a set number of cells, and then determine the suitable incubation time for color development. Below, the method and conditions for using Cell Counting Kit-8 are described.

Procedure	Precautions & Tips
Recover the cells to be assayed from a culture flask. Adjust the concentration of the cell suspension to $5x10^4$ cells per ml using a hematocytometer or cell counter.	For adherent cells, recover the cells using trypsin to detach cells, and use a cell scraper if necessary.
Perform Serial Dilution by… a. Using an 8 channel multi-pipette, add 100 µl of media to each well of a 96 well microplate. b. Add 100 µl of a $5x10^4$ cells per ml solution to the wells of first triplicate row. c. Take 100 µl from the first triplicate row, add it to the next well and mix. The process is repeated as indicated in the figure 2. Reserve the final well for the negative control (Blank). This well should contain media only (no cells) for measurement of the background.	Refer to experimental example on the next page for instruction on serial dilution.
Pre-incubate the plate in a CO_2 incubator. Cells in the log (dividing) phase is strongly recommended. <For adherent cells> Pre-incubation is required for 2-4 hours in order to attached the cells to the plate. <For suspension cells or adherent cells that do not need to be adhered> Pre-incubation can be skipped. Most cell lines would be in the log phase after 48-72 hours of pre-incubation.	
Add 10 µl of Cell Counting Kit-8 reagent to each well on the 96-well microplate.	When using a plate or petri dish other than a 96 well plate, please add reagent equal to 1/10 the media volume. Due to the low volume of reagent added, it is recommended to place the pipette tips against the well wall to add the reagent. If the reagent sticks to the well wall, tap the plate lightly to mix with the media.
Place in a CO_2 incubator for 1-4 hours to react.	If performing the experiment for the first time, we recommend taking readings every hour at 1, 2, 3 and 4 hours.
Measure an absorbance on a microplate reader using a 450 nm filter.	Since the amount of formazan produced will differ with each cell types, the amount of coloration will differ even if the time between adding the reagent and taking a reading is the same. (See HeLa cell and HL60 cell charts on the next page)
Establish your standard curve by plotting the number of cells on the x -axis and the absorbance on the y-axis.	Since bubbles can cause an error, make sure there are no bubbles in the each well. If there are bubbles, use a pipette tip to remove or use a toothpick to pop them.

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Experimental Example

Make serial dilutions of 2.5 x 10^4 , 1.25 x 10^4 , 6.2 x 10^3 ….0 cells per well to each well of a 96 well plate using HeLa cells (human cervical cancer cells) or HL60 cells (promyelocytic leukemia cells) suspensions as indicated in Fig. 2.

Serial Dilution Procedure

Using an 8 channel multi-pipette, add 100 µl of media to each well of a 96 well microplate. Next, add 100 µl of a 5 x 10^4 cells/ml solution to the first well and pipette to mix. This well will have the maximum number of cells. Next, take 100 µl from the first well and add it to the next well, and mix. The process is repeated as indicated in the figure. Take 100 µl from the last well which contains the minimum number of cells and discard.

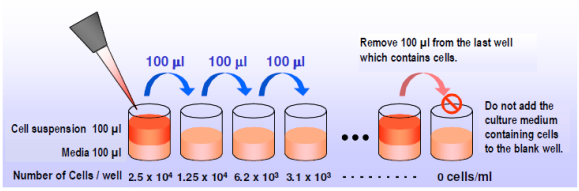


Fig. 2 Serial dilution process

Even when the cell number is the same, HeLa cells (Fig. 3) and HL60 cells (Fig. 4) have quite different cell activities. So, in a preliminary experiment, it is recommended to determine the suitable concentration of cells for each cell type and the time of coloration. In addition, for experiments involving drugs, consider the drug's properties which increases the cell proliferation, cell toxicity, reducing activity, and exposure time to drugs.

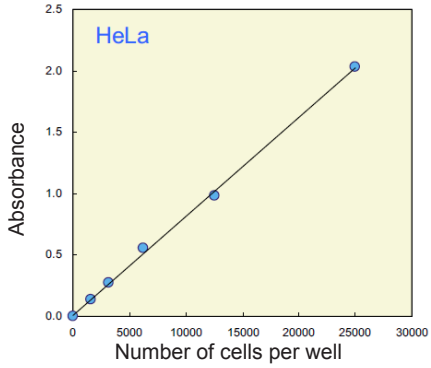


Fig. 3 Color development using HeLa cells
Media: DMEM (10% FBS)
Incubation: 37 °C, 3 hr, 5% CO_2 incubator
Measurement Wavelength: 450nm

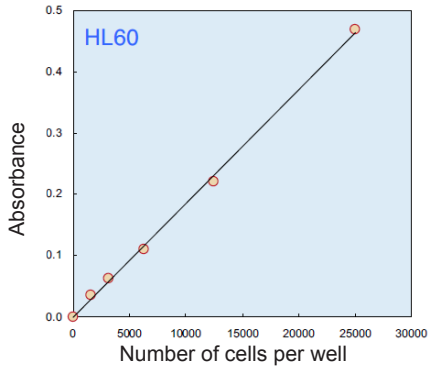


Fig. 4 Color development using HL60 cells
Media: RPMI1640 (10% FBS)
Incubation: 37 °C, 3 hr, 5% CO_2 incubator
Measurement Wavelength: 450nm

As indicated in Fig. 5, there is a good correlation between the Cell Counting Kit-8 assay and [^{3}H]-Thymidine Uptake assay.

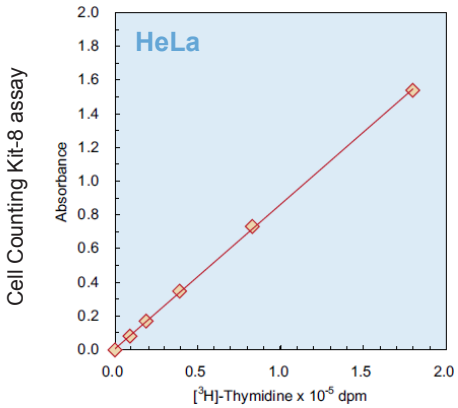
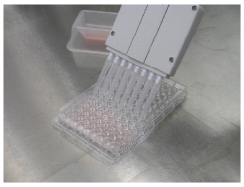
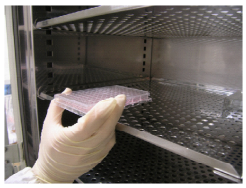
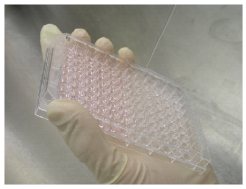
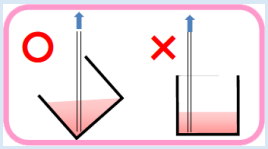
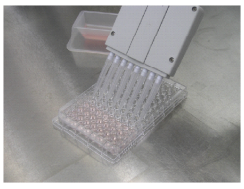
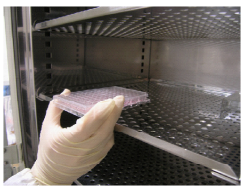


Fig. 5 Correlation between CCK-8 assay and [^{3}H]-Thymidine Uptake assay

Cell Proliferation and Cytotoxicity Protocol

Procedure	Precautions & Tips
Recover the cells to be assayed from a culture flask.	For adhesive cells, recover the cells using trypsin, and use cell scrapers if necessary.
Count the cells and adjust the concentration to desired cell numbers* of the cell suspension. (cell conc.: _____cells/ml)	Use a hemacytometer or a cell counter. *Please see "Optimization of Assay Condition" on Page 6.
Add 100 µl of a cell suspension to each well in a 96 well microplate using serial dilution. Make a well of only media to measure the background. 	For floating type cells, please use a V bottom plate. The upper limit for the microplate reader may be surpassed if too many cells are present. The concentration of the cell should be adjusted based on whether the cells are promoted or inhibited by the test materials.
Incubate for 24-48 hrs. in a CO_2 incubator (start time: _____ end time: _____) 	If the time from starting incubation to taking a measurement is over 48 hrs, it is necessary to exchange the media.
If media change is necessary, remove media and add 100 µl of new media to each well including wells for a background measurement. 	Tilt the plate when removing the media to avoid touching the cells with the tip of the pipette as shown in the below diagram.  For floating type cells, centrifuge a V bottom plate with a microplate rotor, and then remove the media after the cells settle out of the solution with care not to suck in cells.
Add 10 µl of media containing different concentrations of the test substances to each well. 	Add the same amount of test substance to the blank wells (no cells) to measure the background absorbance. For negative control, add 10 µl of media to a well that does not contain the test substance. For dissolving the test substance, it is possible to use PBS or saline solution other than media.
Incubate for set periods of (6, 12, 24, 48 hrs) in a CO_2 incubator. (start time:_____ end time:_____) 	The exposure time depends on the test substance and purpose of the experiment. If the substance is highly toxic to the cell and destroys the cell membrane or reacts directly with proteins or DNA, short exposure time will be appropriate. If the substance slowly affects cell function, longer exposure time may be appropriate.

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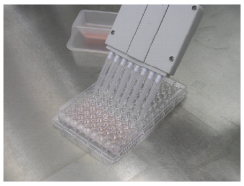
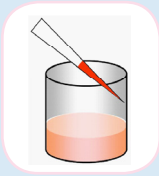
Measuring Cell Viability/Cytotoxicity: Cell Counting Kit-8

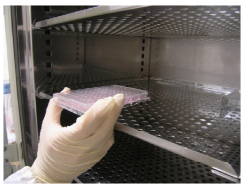
Add 10 µl of Cell Counting Kit-8 to each well in a 96 well microplate.



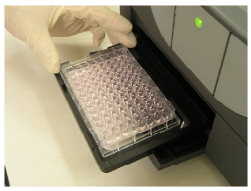
When using a plate or petri dish other than a 96 well plate, please add reagent equal to 1/10 the media volume.
Due to the low volume of reagent added, it is beneficial to touch the tip of the pipette to the well of the wall and when adding the reagent as indicated the figure below. If the reagent sticks to the well wall, tap the plate lightly to mix with the media.



Place in a CO_2 incubator for 1-4 hours to react.
(start time: _____ end time: _____)



Measure the absorbance at 450 nm with a microplate reader.



Since bubbles can cause error, make sure there are no bubbles in each well. If there are bubbles, use a pipette tip to remove or use a toothpick to pop them.

Calculating the Cell Survival Rate

Enter the absorbance reading from each well in the equation below to calculate the cell survival rate.

$$\text{Survival rate (\%)} = \frac{A_{\text{sample}} - A_b}{A_c - A_b} \times 100$$

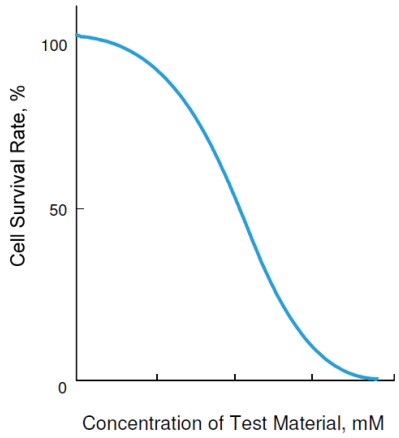


Fig. 6 Typical cell survival curve.

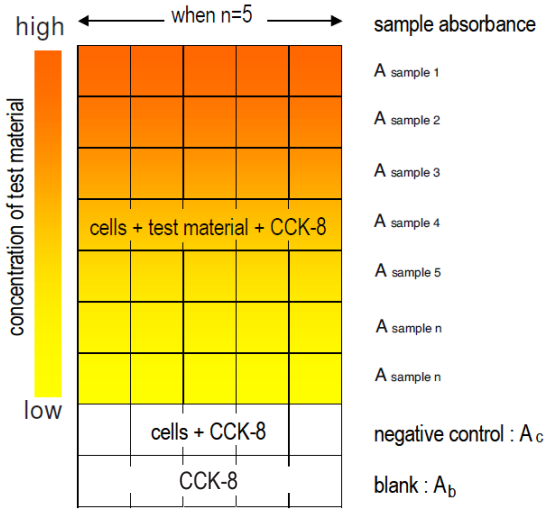


Fig. 7 Example of the plate arrangement and color development.

Does reducing material interfere with the Cell Counting Kit-8 assay?

On occasion when using the Cell Counting Kit-8 for cytotoxcity tests, cells that have been treated with the test material and should be dead may seem to show coloration. In this case, the test material is showing signs of reducing properties and it is possible that it has reduced the WST-8. So, before beginning your experiment, mix the test material and Cell Counting Kit-8 using media to confirm that the material does not react with Cell Counting Kit-8. If there is a significant coloration after the incubation, remove the media and wash the cells with media or PBS (-) to remove the test substance and add the same volume of fresh media to each well prior to adding Cell Counting Kit-8 solution.

Troubleshooting

Problem	Possible Cause	Solution
The absorbance reading exceeds the upper limit of the machine.	Too many cells per well.	The number of viable cells may increase during the pre-incubation. Prepare a microplate with a lower number of cells for the assay. For each cell type, determine the relationship between cell number and O.D readings (please refer to " Optimization of Assay Condition" on page 6).
	Too long of an incubation time.	Shorten the incubation time.
The color development occurs even though cells are clearly dead.	WST-8 is reduced by the test substance or materials which are generated in the culture media during the assay.	Mix Cell Counting Kit-8 with the substance to test whether the substance reacts with the Cell Counting Kit-8. If there is coloration, follow either of the following procedure: 1) Before adding Cell Counting Kit-8, exchange the culture media to remove the test substance or materials. 2) Use Cell Counting Kit-F.
The absorbance of the well with a toxic substance is higher than that of the well with no substance.	Toxic substances in low concentrations sometimes stimulate cell activity. Since cells have functions to protect themselves from the exposure of toxic substances, enzymatic activity of cells may increase at the initial stage. Then, the cell starts to die after a certain concentration.	If determining the LD_{50} of the substance, just ignore the area of increased absorbance.
		Try another method, such as Cell Counting Kit-F, to determine toxicity of the substance.
There is a high variation in the data.	The assay condition of the outer-most wells has changed due to the edge effect.	Do not use the outer-most wells for the assay. Just add media to these wells.
	Cell Counting Kit-8 has not been mixed well with the media.	Lightly tap the outside of the well in order to get the Cell Counting Kit-8 that is on the well wall to fall into the media.
	There are bubbles on the surface of the media.	Remove the bubbles using a pipet tip or a toothpick.
No color or less color development even though the number of cells seems to have increased.	Cell viability of each cell has been lowered because of too many cells.	Reduce the number of cells.

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Q&A

Questions about reagents used in the kit

Q: *What causes color development in Cell Counting Kit-8?*
A: WST-8 is reduced to an orange-colored formazan through electron mediator, 1-Methoxy PMS by NADH and NADPH activity which are generated by cellular activities as indicated in the Fig. 8. The amount of WST-8 formazen is dependent on the activity of cellular dehydrogenase, so WST-8/1-Methoxy PMS system can be used to determine the number of living cells and cell viability.

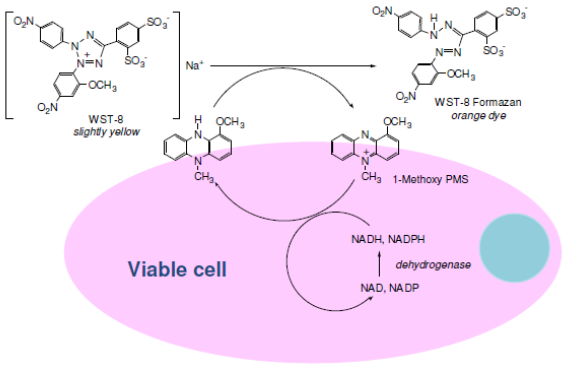

Fig. 8 Cell viability detection mechanism with CCK-8

Q: *Do WST-8 and 1-Methoxy PMS molecules enter into the cell?*
A: There is no clear evidence that these molecules do or do not enter the cell. Generally, a neutrally or positively charged organic molecule such as MTT can enter the cell. Therefore, it is estimated from their charges that 1-Methoxoy PMS can enter the cell, but WST-8 cannot. It is speculated that 1-Methoxy PMS receives an electron from NADH or NADPH at the membrane or inside of the cell and passes the electron to the WST-8 that is around the outer cell membrane.

Q: *What is the stability of the Cell Counting Kit-8?*
A: The Cell Counting Kit-8 is stable for over 6 months at ambient temperature. The kit is stable for over one year when stored in a refrigerator and over two years when stored in a freezer.

Q: *What is the toxicity of Cell Counting Kit-8 compared to MTT?*
A: Compared to MTT in which the cell cannot survive after the reagent has been added, the cell survival rate for Cell Counting Kit-8 is over 90% even after 24 hours incubation. Therefore, after the assay with Cell Counting Kit-8, those cells can be used for another experiments. However, it is necessary to wash the cells so that no dye remains on the cell surface.

Questions regarding cells and cell culture

Q: *What type of cells can be measured using Cell Counting Kit-8?*
A: Generally, Cell Counting Kit-8 can be used for mammalian cell lines, primary culture animal cells, and stem cells.

Q: *How long of a pre-incubation time is required prior to the assay?*
A: It depends on the cell type, but the cells should enter into the logarithmic growth phase. The average incubation time to enter into this phase is 24 hours to 48 hours. Please check cell databases to estimate the pre-incubation time.

Q: *Can Cell Counting Kit-8 be used for both adherent cells and suspension cells?*
A: Yes, it can be used for both types of cells. However, the color development for suspension cells will be lower compared to the coloration of the adherent cells, so it may be necessary to increase the incubation time or increase the number of cells for the assay using non-adherent cells.

Q: *When using Cell Counting Kit-8, what number of cells is appropriate?*
A: The appropriate number of cells depends on the type of cells and the type of your experiment. The amount of coloration will differ depending on the cell type, even if the cell number per well and incubation times are the same. When using a 96 well microplate, please check the absorbance level of 1,000-25,000 cells/well. If the experiment is for toxicity tests, 5,000-10,000 cells/well may be appropriate. If the number of cells are expected to increase during the assay, prepare a plate with 1,000-5,000 cells per well.

Q: *Is it necessary to pre-incubate?*
A: It is recommended to pre-incubate the adherent cells. When collecting the cells from a culture flask using Trypsin, the activity of the cells is not normal. Because of this, it is necessary to pre-incubate to get the cells back to their logarithmic growing phase to regain the viability prior the assay. For non-adherent cells, you can skip this step if the same culture medium is used for harvesting and resuspending cells for the assay.

Questions concerning the assay

Q: *Is it possible to do the assay in a 24 or 12 well plate? If so, how much Cell Counting Kit-8 solution should be used?*
A: Yes, it is possible to assay using plates other than a 96 well plate. Please add Cell Counting Kit-8 solution equal to 1/10 of the volume of the media (if the media is 1 ml, add 100 μl of solution)

Q: *What should be done to stop the color development reaction?*
A: Follow one of the below methods (volume is based on 96 well plates)

Method A) Add 10 μl of 1 % SDS (dissolve 0.1 g SDS with PBS buffer to prepare 10 ml solution)
Notes: Since bubbles on the surface causes errors when measuring the absorbance, be careful not to make bubbles when adding the SDS solution.

Method B) Add 10 μl of 0.1 mol/l acid such as Hydrochloric acid.
Notes: Be sure to take a reading within 24 hrs after stopping the reaction.
When using a media with a high buffering capacity, use a higher concentration of hydrochloric acid to stop the reaction.
Do not use alkaline solution to stop the color development reaction. WST-8 and other tetrazolium salts are not stable under alkaline condition.

Q: *How much incubation time is sufficient for color development?*
A: In general, the incubation time is 1-4 hrs. However, the absorbance will differ between cell types even if the number of cells/well and coloration time are the same. Set an appropriate incubation time to give a proportional relationship between the cell number and the absorbance.

Q: *Are there any materials that can affect the color development when using the Cell Counting Kit-8?*
A: Reducing agents and materials with reducing activity may react with WST-8 and give a false reading. If the material is considered having reducing activity, mix the material solution with Cell Counting Kit-8 and incubate to check whether the material reacts with WST-8. If the material reacts with WST-8, remove the culture medium containing such material from cells and add new culture medium prior to adding Cell Counting Kit-8. Dye materials with absorbance around 450-490 nm will affect the reading. However, absorbance from such dyes can be subtracted as a blank. Such absorbance can be subtracted as a blank and does not affect assay data. For more detailed information, please refer to the following Q&A.

Q: *The cell culture is not clear, and has some turbidity.*
A: Measure the absorbance at 600-650 nm of the well as a reference. Then, the absorbance at 600-650 nm should be subtracted from the absorbance of the same well measured at 450 nm to eliminate the background that comes from turbidity.
Notes: If the turbidity comes from contamination, such as bacteria of fungi, just discard the plate and check the entire cell culture and the plate during the preparation process.

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Measuring Cell Viability/Cytotoxicity: Cell Counting Kit-8

Q: *The cell culture in the well contains a material which has an absorbance around 450 nm, what should I do?*
A: Prepare the well for background measurement which contains all materials except for cells. Measure the background absorbance of the well at 450 nm, and then subtract the background absorbance from the absorbance of the sample well containing all materials and cells.
Notes: If the background absorbance from the material is too high to subtract, remove the culture medium and wash cells with fresh media and add the same volume of fresh media to the well prior to adding the Cell Counting Kit-8.

Q: *What should be done regarding materials that may increase the color development and interfere with the Cell Counting Kit-8 assay?*
A: Determine whether the material interferes with the assay. Add the Cell Counting Kit-8 to the solution which contains the material and incubate for a general assay period.
a)If there is no color development during the incubation, the material does not react with the Cell Counting Kit-8.
b)If there is color development during the incubation, the material does react with the Cell Counting Kit-8. Prepare a couple of wells for background absorbance measurement which contains all material except for cells. Measure the absorbance of the background well at 450 nm and subtract the background from the absorbance of the wells containing all materials and cells.
Notes: If the color development is too high to subtract, remove the culture media, wash the cells with fresh media, and then add the same volume of fresh media to the well prior to adding the Cell Counting Kit-8.

Q: *What should be done regarding materials which may inhibit the color development and interfere with the Cell Counting Kit-8 assay?*
A: Determine whether the material intereferes with the assay. Prepare 0.5 mM NADH solution with PBS. Prepare a couple of wells with and without the material solution. Add 10 μl of 0.5 mM NADH solution and 10 μl of the Cell Counting Kit-8 solution sequentially. Incubate the plate for 10 to 30 min.
a) If both wells with and without the material solution have the same absorbance, the material does not inhibit. Use the material for the assay without modification of the assay protocol.
b) If the well containing the material solution is lower than that of the well without the material solution, remove the culture media and wash the cells with fresh media and add the same volume of fresh media to the well prior to adding the Cell Counting Kit-8.

Q: *What should be done regarding test material which is a reducing agent?*
A: Determine whether the reducing agent interferes with the assay. Add the Cell Counting Kit-8 to the solution containing the reducing material and incubate for a general assay period.
a) If there is no color development during the incubation, the material does not react with the Cell Counting Kit-8.
b) If there is color development during the incubation, the material does react with the Cell Counting Kit-8. Prepare a couple wells which contains all materials except cells for a background absorbance measurement. Measure the absorbance of the background well at 450 nm and subtract the background from the absorbance of the wells that containing all materials and cells.
Notes: If the color development is too high to subtract, remove the culture media and wash the cells with fresh media and add the same volume of fresh media to the wells prior to adding the Cell Counting Kit-8.

References

Origin	Cell Line	References
bovine brain microvascular endothelial	BBMVEC	T. Kitamuro, *et al., J. Biol. Chem.*, **278**, 9125 (2003)
dog canine kidney epithelial cell	MDCK	H. Shimura, *et al., Cancer res.*, **61**, 3640 (2001)
human anaplastic thyroid carcinoma	ARO	F. Furuya, *et al., Endocrinology*, **145**, 2865 (2004)
human B lymphoid	WSU-CLL	M. Ho, *et al., J. Biol. Chem.*, **280**, 607 (2005)
human bladder carcinoma	T24	Y. Shibata, *et al., J. Biol. Chem.*, **277**, 746 (2002)
human bone marrow mesenchymal stem cell	BMMSC	M. Miura, *et al., Stem Cells*, **24**, 1095 (2006)
human bronchial epithelial cell	BEAS-2B	C. A. Reilly, *et al., Toxicol. Sci.*, **73**, 170 (2003)
		M. E. Johansen, *et al., Toxicol. Sci.*, **89**, 278 (2006)
human burkitt lymphoma	Daudi	M. Ho, *et al., J. Biol. Chem.*, **280**, 607 (2005)
	Ramos	M. Ho, *et al., J. Biol. Chem.*, **280**, 607 (2005)
human cervical carcinoma	C33A	W. Yang, *et al., Mol. Cancer Ther.*, **5**, 1610 (2006)
human colon carcinoma	HCT116	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human colorectal adenocarcinoma	DLD-1	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human embryonal carcinoma	NT2N	J. Tessier, *et al., Infect. Immun.*, **75**, 1895 (2007)
human epithelial carcinoma cell	A431	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human esophageal squamous cell carcinoma	KYSE	I. Imoto, *et al., Cancer Res.*, **61**, 6629 (2001)
		K. Nakakuki, *et al., Carcinogenesis*, **23**, 19 (2002)
human gastric cancer cell	SH10TC	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human gingival fibroblast	Gin-1	R. Takii, *et al., Infect. Immun.*, **73**, 883 (2005)
human glioblastoma	T98G	T. Kitamuro, *et al., J. Biol. Chem.*, **278**, 9125 (2003)
	U87MG	S. Kim, *et al., Clin. Cancer Res.*, **11**, 5965 (2005)
		S. Kim, *et al., Clin. Cancer Res.*, **12**, 5550 (2006)
human intrahepatic bile duct carcinoma cell	HuCCT1	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human kidney carcinoma	293T	H. Fuda, *et al., J. Lipid Res.*, **48**, 1343 (2007)
human leukemia cell	Kasumi-1	G. Zhou, *et al., Blood*, **109**, 3441 (2007)
human lung adenocarcinoma	LK87	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human lung cancer cell	H1299	H. Tominaga, *et al., Anal. Commun.*, **36**, 47 (1999)
		S. Semba, *et al., J. Biol. Chem.*, **281**, 28244 (2006)
human lymphoblast cell	SupT1	J. Melton, *et al., J. Biol. Chem.*, **279**, 14315 (2004)
	T-cell	I. Y. Lee, *et al., J. Immunol.*, **175**, 1658 (2005)
	Namalwa	M. Ho, *et al., J. Biol. Chem.*, **280**, 607 (2005)
human medulloblastoma	Daoy	X. Li, *et al., Mol. Cancer Ther.*, **4**, 1912 (2005)
		S. Kim, *et al., Clin. Cancer Res.*, **12**, 5550 (2006)
human mesenchymal stem cell	hMSC	D. Huang, *et al., FASEB J.*, **19**, 2014 (2005)
		L. Song, *et al., Stem Cells*, **24**, 1707 (2006)
human monoblastic lymphoma	U937	R. Hori, *et al., J. Biol. Chem.*, **277**, 10712 (2002)
human multiple myeloma	AMO1	J. Inoue, *et al., Am. J. Pathol.*, **165**, 71 (2004)
	KMS-11	J. Inoue, *et al., Am. J. Pathol.*, **165**, 71 (2004)
human neural stem cell	HB1.F3	S. Kim, *et al., Clin. Cancer Res.*, **11**, 5965 (2005)
		S. Kim, *et al., Clin. Cancer Res.*, **12**, 5550 (2006)
human neuroblastoma	IMR32	H. Tominaga, *et al., Anal. Commun.*, **36**, 47 (1999)
	SK-N-SH	Y. Wang, *et al., J. Virol.*, **78**, 7916 (2004)

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Origin	Cell Line	References
human neuroblastoma	SMS-KAN	A. Misawa, *et al., Cancer Res.*, **65**, 10233 (2005)
human non-small-cell lung cancer cell	LCSC#2	H. Ishibashi, *et al., Cancer Res.*, **65**, 6450 (2005)
	RERF-LC-OK	H. Ishibashi, *et al., Cancer Res.*, **65**, 6450 (2005)
human ovarial cancer cell	OVK18	H. Ohori, *et al., Mol. Cancer Ther.*, **5**, 2563 (2006)
human ovarian adenocarcinoma	HTOA	M. Furuya, *et al., Cancer Res.*, **65**, 2617 (2005)
human pancreatic cancer cell	Alexander cell	S. Awale, *et al., Cancer Res.*, **66**, 1751 (2006)
	AsPC-1	T. Mori, *et al., Mol. Cancer Ther.*, **3**, 29 (2004)
		S. Awale, *et al., Cancer Res.*, **66**, 1751 (2006)
	BxPC-3	S. Awale, *et al., Cancer Res.*, **66**, 1751 (2006)
	MiaPaCa-2	A. Aghdassi, *et al., Cancer Res.*, **67**, 616 (2007)
	PBMC	T. Mori, *et al., Mol. Cancer Ther.*, **3**, 29 (2004)
		S. Awale, *et al., Cancer Res.*, **66**, 1751 (2006)
		A. Aghdassi, *et al., Cancer Res.*, **67**, 616 (2007)
human peripheral blood mononuclear cell	PBMC	C. Chang, *et al., Stem Cells*, **24**, 2466 (2006)
		T. Lee, *et al., Mol. Cancer Ther.*, **5**, 2398 (2006)
human prostate carcinoma	LNCaP	D. J. Son, *et al., Mol. Cancer Ther.*, **6**, 675 (2007)
human pulmonary adenocarcinoma	H441	H. Shimura, *et al., Cancer res.*, **61**, 3640 (2001)
human skin mast cell	primary Mast cell	J. Tessier, *et al., Infect. Immun.*, **75**, 1895 (2007)
human T cell	Jurkat	T. Kasai, *et al., J. Biol. Chem.*, **277**, 5187 (2002)
		L. Lu, *et al., J. Biochem.*, **141**, 157 (2007)
mouse cortical neurons, primary		M. Ikonen, *et al., PNAS*, **100**, 13042 (2003)
mouse Macrophage		Y. Miyake, *et al., J. Immunol.*, **178**, 5001 (2007)
mouse embryonic fibroblast	Balb3T3	H. Tominaga, *et al., Anal. Commun.*, **36**, 47 (1999)
	3T3-L1	D. Huang, *et al., FASEB J.*, **19**, 2014 (2005)
mouse fibroblast	L929	H. Tominaga, *et al., Anal. Commun.*, **36**, 47 (1999)
	NIH3T3	R. Yu, *et al., Toxicol. Sci.*, **93**, 82 (2006)
mouse hippocampal cell	HT22	H. Sohn, *et al., FASEB J.*, **20**, 1428 (2006)
mouse insulinoma	MIN6	S. Oyadomari, *et al., PNAS*, **98**, 10845 (2001)
mouse macrophage	RAW 264	M. Shiga, *et al., Anesth. Analg.*, **92**, 128 (2001)
	RAW 264.7	S. Oyadomari, *et al., PNAS*, **98**, 10845 (2001)
		D. J. Son, *et al., Mol. Cancer Ther.*, **6**, 675 (2007)
mouse hepatocellular carcinoma	MH134	S. Shibata, *et al., J. Immunol.*, **177**, 3564 (2006)
mouse malignant melanoma	B16F1	S. Shibata, *et al., J. Immunol.*, **177**, 3564 (2006)
Wistar rats calvaria	osteoblast	E. Hinoi, *et al., FASEB J.*, **17**, 1532 (2003)

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