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# Dojindo Cell Counting Kit-8 Handbook

# Dojindo Cell Counting Kit-8 Assay

Procedure for use with Dojindo CCK-8;
Catalog #: CK04

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# Dojindo Cell Counting Kit-8 Assay

## Simple Procedure for Cell Preparation, Proliferation and Cytotoxicity

## Introduction

Cell viability and cytotoxicity assays are often used for drug screening and chemical cytotoxicity tests. Dojindo's Cell Counting Kit-8 utilizes highly water-soluble tetrazolium salts, called WSTs, to produce water-soluble formazans which are suitable for cell proliferation and cytotoxicity assays. Unlike MTT, WST-8 and WST-8 formazan have no cytotoxicity in cell culture media which allows for multiple, downstream experiments to be carried out using the same assay plate. The Dojindo CCK-8 is a convenient colorimetric assay for the determination of cell viability, requires no dissolving or solubilizing process and provides results with minimal steps. CCK-8 can be used in 96-well microplate assays as well as high throughput screening 384-well microplate assays.

## Materials

- Dojindo's Cell Counting Kit-8 (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

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

## Method

### Cell Preparation and Standard Curve

1. Recover the cells to be assayed from a culture flask.
    a. For adherent cells, recover using trypsin to detach cells, and use a cell scraper if necessary.

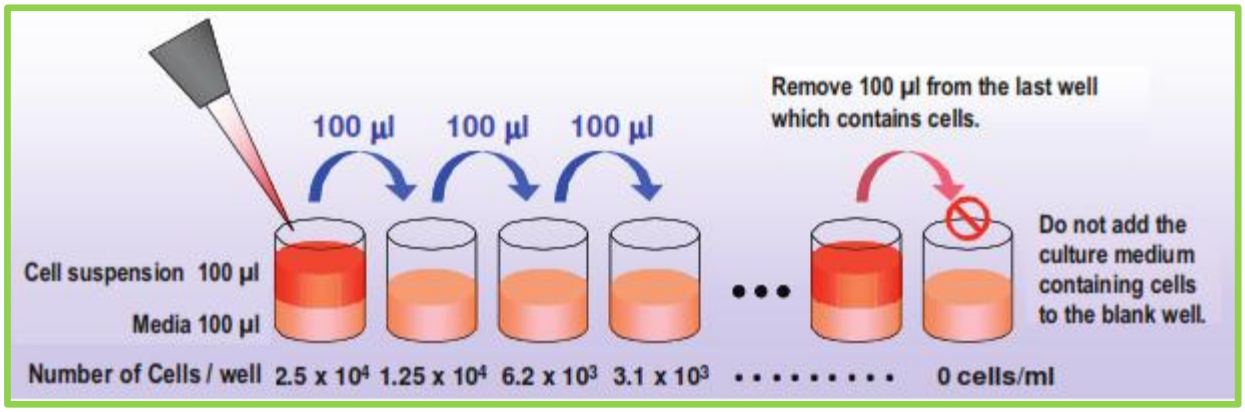
2. Measure the cells and adjust the concentration of the cell suspension using a hematocytometer or cell counter.
    a. Cell suspensions are typically adjusted to $5x10^4$ cells per ml.

3. Using an 8 channel multi-pipette, add 100 µl of media to each well of 96-well microplate.

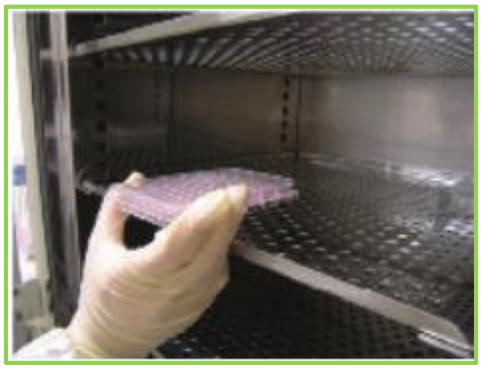
4. Make duplicate or triplicate serial dilutions of $2.5x10^4$, $1.25x10^4$ and $6.25x10^3$ in a 96-well plate.

a. Add 100 µl of $5x10^4$ cells per ml solution to the first well of the plate and pipette to mix. Next, transfer 100 µl of cells from the first well to the second well, mix and then sequentially transfer 100 µl to the next well after mixing the cells. Repeat until you have made serial dilutions of cells in all wells but the final well.

b. Reserve the final well for the negative control. This well should contain media only (no cells) for measurement of the background.



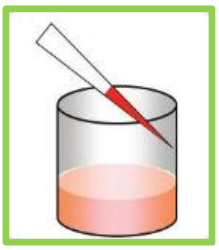

5. Incubate the 96-well microplate for 24-48 hours in a $CO_2$ incubator.
   a. If performing the experiment for the first time, we recommend that both a 24 and 48 hour experiment be performed to determine the optimal growth time for the standard curve.



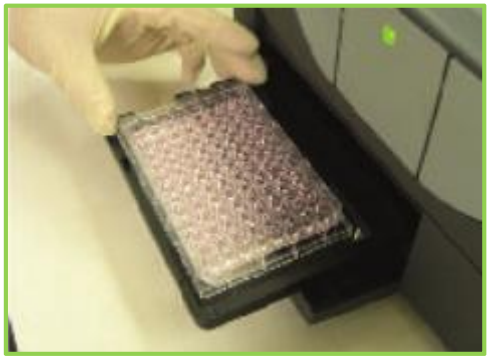
6. Add 10 µl of Cell Counting Kit-8 reagent (one tenth of the cells + media) to each well on the 96-well microplate.

   **NOTE: Due to the low volume of reagent added, it is recommended to touch the tip of the pipette to the well of the wall when adding the reagent. If the reagent sticks to the well wall, tap the plate lightly to mix with the media.**

   **NOTE: When using a plate or petri dish other than a 96-well plate, add reagent equal to 1/10 the media volume.**



7. Place in a $CO_2$ incubator for 1-4 hours to react.
    a. If performing the experiment for the first time, we recommend taking readings at 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 hours.
    b. Take a colorimetric reading on a microplate reader using a filter for 450 nm. This experiment will help to establish a standard curve and CCK-8 incubation time specific to your cell type.



8. Plot the number of cells versus the absorbance in order to establish your standard curve.

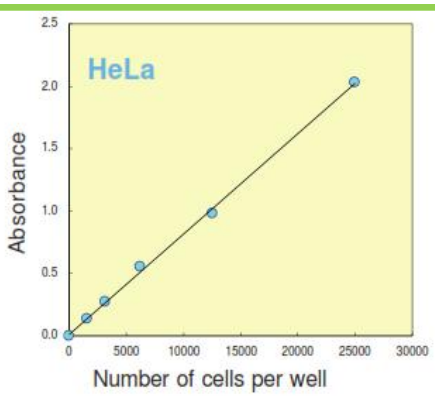



Fig. 3 Color development using HeLa cells
Media: DMEM (10% FBS)
Incubation: 37 °C, 3 hr, 5% $CO_2$ incubator
Measurement Wavelength: 450nm

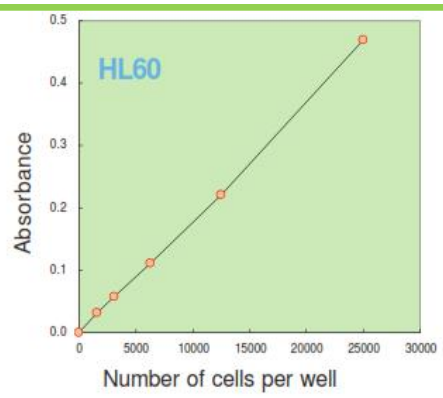



Fig. 4 Color development using HL60 cells
Media: RPMI1640 (10% FBS)
Incubation: 37 °C, 3 hr, 5% $CO_2$ incubator
Measurement Wavelength: 450nm

**Cell Proliferation and Cell Toxicity Protocol**

1. Recover the cells to be assayed from a culture flask.
    a. For adherent cells, recover using trypsin to detach cells, and use a cell scraper if necessary.

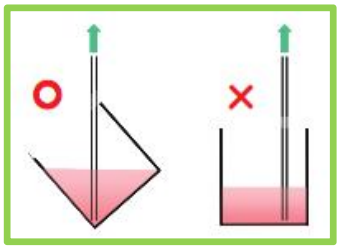
2. Measure the cells and adjust the concentration to desired cell numbers of the cell suspension as determined from the standard curve (See: Cell Preparation and Standard Curve).
    a. The number of cells used should be in the upper portion (80%) of the linear range of the standard curve*. This allows for significant absorbance measurements with more toxic substances.

    * The number of cells may be adjusted depending upon your test substance.

3. Add 100 µl of the cell suspension to each well of a 96-well microplate.

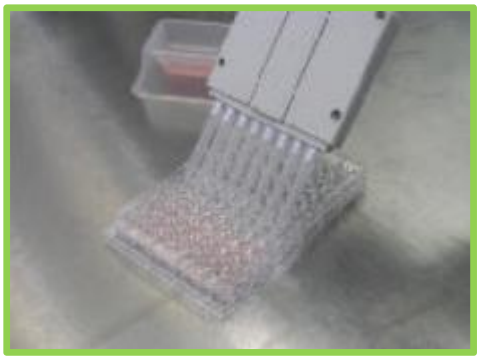
a. Reserve the final well for the negative control. This well should contain media only (no cells) for measurement of the background.

4. Incubate the 96-well microplate for 24-48 hours in a $CO_2$ incubator.
   a. It may be necessary to change media. Remove media with a micropipet or a Pasteur pipette and add 100 µl of fresh media to each well including wells for the background negative control 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.**

5. Add 10 µl of media containing different concentrations of the test substance to each well.
   **NOTE: 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.**



6. Incubate for set periods (6, 12, 24, 48 hours) in a $CO_2$ incubator.
   a. 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.

7. Add 10 µl of Cell Counting Kit-8 reagent (one tenth of the cells + media) to each well on the 96-well microplate.

8. Place in a $CO_2$ incubator for the 1-4 hour time period, the exact time having been determined from the standard curve (See: Cell Preparation and Standard Curve).
   a. This time period will be specific to your cell type and may be adjusted depending upon the toxicity of your test substance. Take a colorimetric reading on a microplate reader using a filter for 450 nm.

## Calculations

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

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

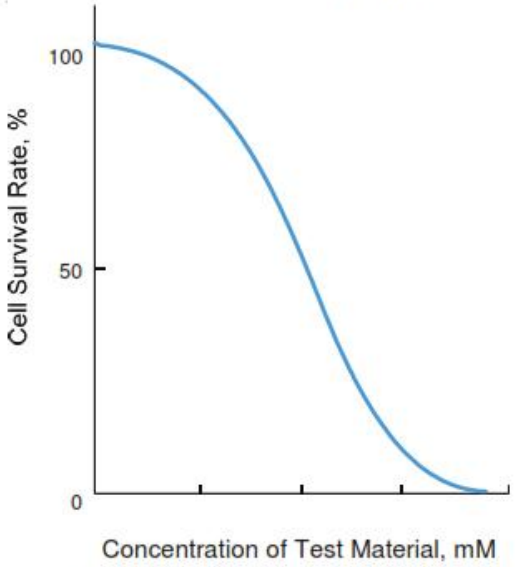



Fig. 6 Typical cell survival curve.

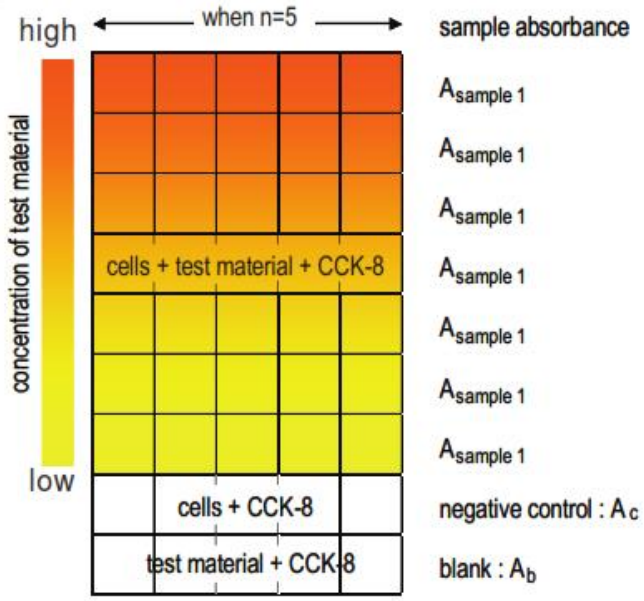



Fig. 7 Example of the plate arrangement and color development

## Tips

- Bubbles may cause errors on the plate reader. Make sure that all wells are free of bubbles. If there are bubbles, use a pipette tip to remove or a toothpick to pop them.
- 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, give consideration to the drug's properties such as enhancing cell proliferation, toxicity to cells, and reducing activity, in addition to time of exposure to drugs.
- The upper limit for the microplate reader may be surpassed if too many cells are present.
- For floating type cells, it is best to use a V bottom plate. To exchange media from a floating type cell culture, centrifuge the V bottom plate with a microplate rotor at a low speed (50-100 x g). After centrifugation, remove the media the solution with care to avoid disturbing or removing any cells.

- For dissolving the test substance, it is possible to use PBS or saline solution other than media.

- Occasionally 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 have reducing properties and it is possible that it has reduced the WST-8. 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 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 the Cell Counting Kit-8.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Absorbance reading exceeds the upper limit of the machine. | Too many cells per well. | The number of viable cells may increase during the preincubation. 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 (Refer to Cell Preparation and Standard Curve). |
| | Too much incubation time. | Shorten the incubation time. |
| The absorbance is higher than that of the well with no substance when a toxic substance is added to the cell. | 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, ignore the area of increased absorbance. |
| 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 for the assay. |

| Problem | Possible Cause | Solution |
|---|---|---|
| Color development occurs even though cells are clearly dead when using the kit for cytotoxicity assays. | WST-8 is being 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, change the culture media to remove the test substance or materials in the culture media before adding the Cell Counting Kit-8. |
| There is high variation in the data. | The assay condition of the outermost 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. |

## Questions about the reagents in this kit

### What causes color development according to the viable cell number in Cell Counting Kit-8?

WST-8 is reduced to an orange-colored formazan through 1-methoxy PMS by NADH and NADPH which are generated by cellular activities as indicated in Fig. 8. The amount of WST-8 formazan 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.

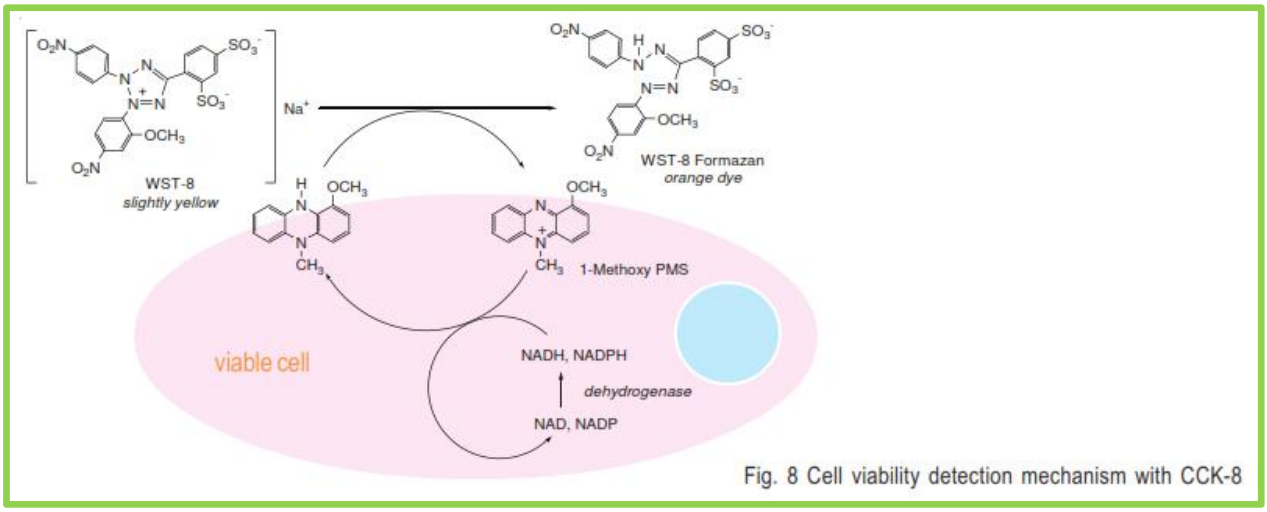



Fig. 8 Cell viability detection mechanism with CCK-8

### Do WST-8 and 1-Methoxy PMS molecules enter into the cell?

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.

### How is the stability of the Cell Counting Kit-8?

The Cell Counting Kit-8 is stable for over 3 months at ambient temperature. Therefore, it is possible to ship this kit without dry ice or blue ice. The kit is stable for over one year when stored in a refrigerator and over two years when stored in a freezer.

### How is the toxicity of Cell Counting Kit-8 compared to MTT?

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. Because of this, after assaying with Cell Counting Kit-8, those cells can be used for other experiments. However, it is necessary to wash the cells so that no dye remains on the cell surface.

## Questions regarding cells and cell culture

### What type of cells can be assayed by Cell Counting Kit-8?

Generally, Cell Counting Kit-8 can be used for animal cell lines and primary culture animal cells.

### How long of a pre-incubation time is required prior to assay?

It depends on the cell type. The cells for the assay should enter into the logarithmic growth phase. The average incubation time to enter into this phase is from 24 hours to 48 hours. Please check cell databases to estimate the pre-incubation time.

### Can Cell Counting Kit-8 be used for both adherent cells and nonadherent cells?

It can be used for both types of cells. However, the color development for non-adherent cells will be low compared to the coloration for adherent cells, so it may be necessary to increase the time for coloration or increase the number of cells for the assay using nonadherent cells.

### When using Cell Counting Kit-8, what number of cells is appropriate?

The number of cells depends on the type of cells and the type of experiment. The amount of coloration will differ depending on cell type, even if the cell number per well and coloration 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/well.

### Is it necessary to pre-incubate?

It is recommended to pre-incubate 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 to use for assays. For nonadherent 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

### 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?

It is possible to assay using plates other than a 96-well plate. Please add Cell Counting Kit-8 solution equal to 1/10 the volume of the media (i.e., if the media volume is 1 ml, add 100 µl of CCK-8 solution).

### What should be done to stop the color development reaction?

Follow one of the below methods (volume is based on 96-well plates):

A. Add 10 µl of 1% SDS (dissolve 0.1 g SDS with PBS buffer to prepare 10 ml solution).
Note: Be careful not to avoid creating bubbles when adding the SDS solution. Bubbles on the surface cause serious error for the measurement of absorbance.

B. Add 10 µl of 0.1M acid such as hydrochloric acid.
Notes: Be sure to take a reading within 24 hours 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.

### How much incubation time is sufficient for color development?

In general, the incubation time is 1-4 hours. 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.

### Are there any materials that can affect the color development from the Cell Counting Kit-8?

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 discover if the material solution reacts with WST-8. If the material is reactive with WST-8, remove the culture medium containing such material from cells and add fresh culture medium, without test material, prior to adding Cell Counting Kit-8. Dye materials with absorbance around 450-490 nm will also affect the reading. Absorbance from such dyes may be subtracted as a blank. For example, Phenol Red has an absorbance near the assay wavelength. Such absorbance can be subtracted as a blank and does not affect assay data. For additional information, please refer the following Q&A.

### The cell culture is not clear. It has some turbidity.

Measure the absorbance at 600-650 nm of the well as a reference. Then, the absorbance at 600-650 nm is 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.

### The cell culture in the well contains material which has an absorbance around 450 nm, what should I do?

Use a couple of wells for a background absorbance measurement to subtract the total absorbance of the sample wells. Prepare the wells 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.

**NOTE: 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.**

### What should be done regarding materials that may increase the color development and interfere with the Cell Counting Kit-8 assay?

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 a background absorbance measurement which contains all material except for cells. Measure the absorbance of the background well at 450 nm and subtract this background from the absorbance of the wells containing all materials and cells.

**NOTE: 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.**

### What should be done regarding materials which may inhibit the color development and interfere with the Cell Counting Kit-8 assay?

Determine whether the material interferes with the assay. Prepare 0.5mM NADH solution with PBS. Prepare a couple of wells with and without the material solution. Add 10 µl of 0.5mM NADH solution and 10 µl of the Cell Counting Kit-8 solution sequentially. Incubate the plate for 10 to 30 minutes.

a) If both wells, with and without the material solution, have the same absorbance, the material does not inhibit the Cell Counting Kit-8 assay. 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.

### What should be done regarding a test material which is a reducing compound?

Determine whether the reducing material 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.

**NOTE:** 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

| Cell Line | Origin | Reference |
|---|---|---|
| **293T** | human kidney carcinoma | H. Fuda, et al., J. Lipid Res., 48, 1343 (2007) |
| **3T3-L1** | mouse embryonic fibroblast | D. Huang, et al., FASEB J., 19, 2014 (2005) |
| **3Y1** | rat | N. Itano, et al., PNAS, 99, 3609 (2002) |
| **A431** | human epithelial carcinoma cell | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **Alexander cell** | pancreatic cancer cell | S. Awale, et al., Cancer Res., 66, 1751 (2006) |
| **AMO1** | multiple myeloma | J. Inoue, et al., Am. J. Pathol., 165, 71 (2004) |
| **ARO** | human anaplastic thyroid carcinoma | F. Furuya, et al., Endocrinology, 145, 2865 (2004) |
| **AsPC-1** | pancreatic cancer cell | T. Mori, et al., Mol. Cancer Ther., 3, 29 (2004)<br>S. Awale, et al., Cancer Res., 66, 1751 (2006) |
| **B16F1** | murine malignant melanoma | S. Shibata, et al., J. Immunol., 177, 3564 (2006) |
| **Balb3T3** | mouse embryonic cell | H. Tominaga, et al., Anal. Commun., 36, 47 (1999) |
| **BBMVEC** | bovine brain microvascular endothelial | T. Kitamuro, et al., J. Biol. Chem., 278, 9125 (2003) |
| **BEAS-2B** | human bronchial epithelial cell | C. A. Reilly, et al., Toxicol. Sci., 73, 170 (2003)<br>M. E. Johansen, *et al.*, *Toxicol. Sci.*, 89, 278 (2006) |
| **BMMSC** | bone marrow mesenchymal stem cell | M. Miura, et al., Stem Cells, 24, 1095 (2006) |
| **BxPC-3** | pancreatic cancer cell | S. Awale, et al., Cancer Res., 66, 1751 (2006) |
| **C33A** | human cervical carcinoma | W. Yang, et al., Mol. Cancer Ther., 5, 1610 (2006) |
| **C8166** | T-cell | T. Kasai, et al., J. Biol. Chem., 277, 5187 (2002) |
| **cardiomyocyte** | rat | E. E. Hamm, et al., PNAS, 103, 14176 (2006) |

| Cell Line | Origin | Reference |
|---|---|---|
| **cerebellar granule neuron** | rat | X. Wang, et al., J. Biol. Chem., 280, 16705 (2005) |
| **cortical neurons, primary** | mouse | M. Ikonen, et al., PNAS, 100, 13042 (2003) |
| **Daoy** | human medulloblastoma | X. Li, et al., Mol. Cancer Ther., 4, 1912 (2005)<br>S. Kim, *et al.*, *Clin. Cancer Res.*, 12, 5550 (2006) |
| **Daudi** | human burkitt lymphoma | M. Ho, et al., J. Biol. Chem., 280, 607 (2005) |
| **DLD-1** | human colorectal adenocarcinoma | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **Gin-1** | human gingival fibroblast | R. Takii, et al., Infect. Immun., 73, 883 (2005) |
| **FRT** | rat thyroid cell | H. Shimura, et al., Cancer res., 61, 3640 (2001) |
| **H1299** | human lung cancer cell | H. Tominaga, et al., Anal. Commun., 36, 47 (1999)<br>S. Semba, *et al.*, *J. Biol. Chem.*, 281, 28244 (2006) |
| **H441** | human pulmonary adenocarcinoma | H. Shimura, et al., Cancer res., 61, 3640 (2001) |
| **HB1.F3** | human neural stem cell | S. Kim, et al., Clin. Cancer Res., 11, 5965 (2005)<br>S. Kim, *et al.*, *Clin. Cancer Res.*, 12, 5550 (2006) |
| **HCT116** | human colon carcinoma | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **hMSC** | human mesenchymal stem cell | D. Huang, et al., FASEB J., 19, 2014 (2005)<br>L. Song, *et al.*, *Stem Cells*, 24, 1707 (2006) |
| **HT22** | mouse hippocampal cell | H. Sohn, et al., FASEB J., 20, 1428 (2006). |
| **HTOA** | human ovarian adenocarcinoma | M. Furuya, et al., Cancer Res., 65, 2617 (2005) |
| **HuCCT1** | human intrahepatic bile duct carcinoma cell | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **IMR32** | human neuroblastoma | H. Tominaga, et al., Anal. Commun., 36, 47 (1999) |
| **ILT-Hod** | T-cell | T. Kasai, et al., J. Biol. Chem., 277, 5187 (2002) |
| **Jurkat** | human T cell | T. Kasai, et al., J. Biol. Chem., 277, 5187 (2002)<br>L. Lu, et al., J. Biochem., 141, 157 (2007) |
| **Kasumi-1** | acute myeloid leukemia cell | G. Zhou, et al., Blood, 109, 3441 (2007) |
| **KMS-11** | multiple myeloma | J. Inoue, et al., Am. J. Pathol., 165, 71 (2004) |
| **KYSE** | esophageal squamous cell carcinoma | I. Imoto, et al., Cancer Res., 61, 6629 (2001)<br>K. Nakakuki, *et al.*, *Carcinogenesis*, 23, 19 (2002) |
| **L929** | mouse fibroblast | H. Tominaga, et al., Anal. Commun., 36, 47 (1999) |
| **LCSC#2** | non-small-cell lung cancer cell | H. Ishibashi, et al., Cancer Res., 65, 6450 (2005) |
| **LK87** | human lung adenocarcinoma | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **LLC-PK1** | porcine renal tubular | T. Yano, et al., Am. J. Pathol., 166, 1333 (2005) |
| **LNCaP** | human prostate carcinoma | D. J. Son, et al., Mol. Cancer Ther., 6, 675 (2007) |
| **Macrophage** | mouse | Y. Miyake, et al., J. Immunol., 178, 5001 (2007) |
| **Mast cell** | human skin mast cell, primary | J. Tessier, et al., Infect. Immun., 75, 1895 (2007) |
| **MDCK** | canine kidney epithelial cell | H. Shimura, et al., Cancer res., 61, 3640 (2001) |
| **MH134** | murine hepatocellular carcinoma | S. Shibata, et al., J. Immunol., 177, 3564 (2006) |
| **MiaPaCa-2** | pancreatic cancer cell | A. Aghdassi, et al., Cancer Res., 67, 616 (2007) |
| **MIN6** | mouse insulinoma | S. Oyadomari, et al., PNAS, 98, 10845 (2001) |

| Cell Line | Origin | Reference |
|---|---|---|
| **MT1** | T-cell | T. Kasai, et al., J. Biol. Chem., 277, 5187 (2002) |
| **Namalwa** | human lymphoblastoid | M. Ho, et al., J. Biol. Chem., 280, 607 (2005) |
| **NS/PC** | neural stem/progenitor cell | W. Jiang, et al., J. Clin. Invest., 115, 3104 (2005) |
| **NIH3T3** | mouse fibroblast | R. Yu, et al., Toxicol. Sci., 93, 82 (2006) |
| **NT2N** | human embryonal carcinoma | J. Tessier, et al., Infect. Immun., 75, 1895 (2007) |
| **osteoblast** | from calvaria of Wistar rats | E. Hinoi, et al., FASEB J., 17, 1532 (2003) |
| **OVK18** | human ovarial cancer cell | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **PANC-1** | pancreatic cancer cell | T. Mori, et al., Mol. Cancer Ther., 3, 29 (2004)<br>S. Awale, *et al.*, *Cancer Res.*, 66, 1751 (2006)<br>A. Aghdassi, *et al.*, *Cancer Res.*, 67, 616 (2007) |
| **PBMC** | human peripheral blood mononuclear cell | C. Chang, et al., Stem Cells, 24, 2466 (2006)<br>T. Lee, *et al.*, *Mol. Cancer Ther.*, 5, 2398 (2006) |
| **PFSK** | primitive neuroectodermal tumour cell | X. Li, et al., Mol. Cancer Ther., 4, 1912 (2005). |
| **PSN-1** | pancreatic cancer cell | S. Awale, et al., Cancer Res., 66, 1751 (2006) |
| **Ramos** | human burkitt lymphoma | M. Ho, et al., J. Biol. Chem., 280, 607 (2005) |
| **RAW 264** | mouse macrophage | M. Shiga, et al., Anesth. Analg., 92, 128 (2001) |
| **RAW 264.7** | mouse macrophage | S. Oyadomari, et al., PNAS, 98, 10845 (2001)<br>D. J. Son, *et al.*, *Mol. Cancer Ther.*, 6, 675 (2007) |
| **RERF-LC-OK** | non-small-cell lung cancer cell | H. Ishibashi, et al., Cancer Res., 65, 6450 (2005) |
| **RP9** | B lymphoblastoid cell | R. A. Dalloul, et al., Poult. Sci., 85, 446 (2006) |
| **SH10TC** | human gastric cancer cell | H. Ohori, et al., Mol. Cancer Ther., 5, 2563 (2006) |
| **SK-N-SH** | human neuroblastoma | Y. Wang, et al., J. Virol., 78, 7916 (2004) |
| **SMS-KAN** | human neuroblastoma | A. Misawa, et al., Cancer Res., 65, 10233 (2005) |
| **SUIT-2** | pancreatic cancer cell | T. Mori, et al., Mol. Cancer Ther., 3, 29 (2004) |
| **SupT1** | human lymphoblast cell | J. Melton, et al., J. Biol. Chem., 279, 14315 (2004) |
| **T-cell** | | I. Y. Lee, et al., J. Immunol., 175, 1658 (2005) |
| **T24** | human bladder carcinoma | Y. Shibata, et al., J. Biol. Chem., 277, 746 (2002) |
| **T98G** | human glioblastoma | T. Kitamuro, et al., J. Biol. Chem., 278, 9125 (2003 |
| **TL-Omi** | T-cell | T. Kasai, et al., J. Biol. Chem., 277, 5187 (2002) |
| **U87MG** | human glioblastoma | S. Kim, et al., Clin. Cancer Res., 11, 5965 (2005)<br>S. Kim, *et al.*, *Clin. Cancer Res.*, 12, 5550 (2006) |
| **U937** | human monoblastic lymphoma | R. Hori, et al., J. Biol. Chem., 277, 10712 (2002) |
| **WSU-CLL** | human B lymphoid | M. Ho, et al., J. Biol. Chem., 280, 607 (2005) |