

Product Information

96992 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
	Too much incubation time.	Shorten the incubation time.
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 with the substance to test whether the substance reacts with the Cell Counting Kit. If there is coloration, follow either of the following: 1) Before adding the Cell Counting Kit, change the culture media to remove the test substance or materials in the culture media. 2) Use Cell Counting Kit
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 LD50 of the substance, just ignore the area of increased absorbance. Try another method, such as Cell Counting Kit to determine toxicity of the substance.

Problem	Possible Cause	Solution
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 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 for the assay.

Questions about reagents used in the kit

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

A: 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 the 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.

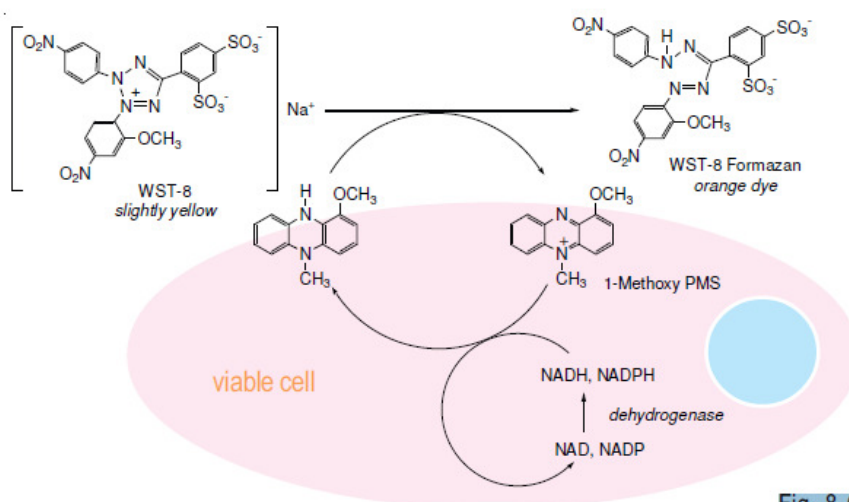


Fig. 8 Cell viability detection mechanism with CCK-8

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-Methoxy 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: How is the stability of the Cell Counting Kit-8?

A: 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.

Q: How 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. 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

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

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

Q: How long of a preincubation time is required prior to assay?

A: 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 preincubation time.

Q: Can Cell Counting Kit-8 be used for both adherent cells and non-adherent cells?

A: 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 non-adherent cells.

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

A: 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.

Q: Is it necessary to preincubate?

A: It is recommended to preincubate 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 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: 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 (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: Be careful not to make bubbles when adding the SDS solution. Bubbles on the surface cause serious error for the measurement of absorbance.

- 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 from 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 or not. Then, 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 affect the reading. However, absorbance from such dyes can 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 more detailed information, please refer the following Q&A.

Q: The cell culture is not clear. It has some turbidity.

A: 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 or fungi, just discard the plate and check the entire cell culture and the plate during the preparation process.

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

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

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.

Precautions and Disclaimer

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