

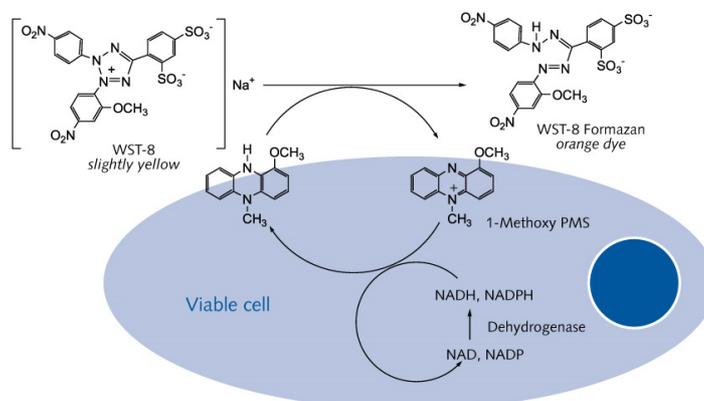
## Instruction Manual

Product Name	Product Description	Size	Catalog Number
Colorimetric Cell Viability Kit I (WST-8)	Colorimetric Cell Viability Kit I (WST-8)	100 assays 500 assays 2500 assays	PK-CA705-CK04-100 PK-CA705-CK04 PK-CA705-CK04-2500

## Introduction

PromoKine's highly sensitive nonradioactive Colorimetric Cell Viability Kit I (CCVK-I, WST-8) allows convenient colorimetric assays using the tetrazolium salt WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces the water-soluble, yellow-colored formazan dye upon bioreduction in the presence of an electron carrier, 1-methoxy PMS (Fig. 1). Colorimetric Cell Viability Kit I (WST-8) is a one-bottle solution: the CCVK-I solution is added directly to the cells; no pre-mixing of components is required. CCVK-I allows sensitive determination of the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. The detection sensitivity of cell proliferation assays using WST-8 is significantly higher than assays using the other tetrazolium salts such as MTT, XTT, MTS or WST-1. Since the CCVK-I solution is very stable and has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.

**Fig. 1: Assay Mechanism**



## Kit Contents

PK-CA705-CK04: 1 bottle (5 ml) CCVK-I solution sufficient for up to 500 tests  
 PK-CA705-CK04-100: 1 bottle (1 ml) CCVK-I solution sufficient for up to 100 tests  
 PK-CA705-CK04-2500 5 bottles (5 ml) CCVK-I solution sufficient for up to 2500 tests  
 - technical manual

## Required Equipments and Materials

- plate reader (450 nm filter)
- 96-well plate
- 10  $\mu$ l, 100-200  $\mu$ l and multi-channel pipettes
- CO<sub>2</sub> incubator

## Storage and Stability

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PromoKine's *CCVK-I Kit* is stable for 2 years at -20°C, 1 year at 4°C and 6 months at room temperature with protection from light. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. To avoid repeated thawing and freezing, keep the kit at 4°C if it is frequently used.

## Assay Protocol

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### General Assay Procedure

1. Prepare a cell suspension with 50,000-100,000 cells/ml using an appropriate culture medium.
2. Add 100 µl of the cell suspension to each well of a 96-well plate.
3. Pre-incubate the plate at 37°C.<sup>a)</sup>
4. Add 10 µl of various concentrations of a solution to be tested<sup>b)</sup> to each well.
5. Incubate the plate at 37°C for a certain time period, such as 24, 48 or 72 hours.
6. Add 10 µl *CCVK-I* solution<sup>c)</sup> to each well, and incubate the plate at 37°C for 1-4 hours.<sup>d)</sup>
7. Read the O.D. at 450 nm to determine the cell viability in each well.
  - a) Overnight preincubation in a CO<sub>2</sub> incubator is recommended.
  - b) Use the culture medium or PBS to prepare the solutions.
  - c) If the solution to be tested has reducing activities, incubate the solution and *CCVK-I* without cells to determine the background absorbance at 450 nm. If the absorbance is negligibly small, add *CCVK-I* solution to each well. If the absorbance is high, remove the culture medium and wash cells twice with the medium, then add 100 µl of the culture medium and 10 µl *CCVK-I* solution.
  - d) Longer incubation may be necessary for leukocyte cells.

### Cell Proliferation Assay

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Also prepare wells that contain known numbers of viable cells (to create a calibration curve in step 5). Pre-incubate the plate in a humidified incubator (e.g., at 37 °C, 5% CO<sub>2</sub>).
2. Thaw the *CCVK-I* on the bench top or in a water bath at 37°C if it is frozen. It takes about 30 minutes on the bench top at 25°C or 5 minutes in a water bath at 37°C.
3. Add 10 µl of the *CCVK-I* solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. Incubate the plate for 1-4 hours in the incubator.
5. Measure the absorbance at 450 nm using a microplate reader. Prepare a calibration curve using the data obtained from the wells that contain known numbers of viable cells. To measure the absorbance later, add 10 µl of 1% (w/v) SDS to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 48 hours.

### Cytotoxicity Assay

1. Dispense 100 µl of cell suspension (5,000 cells/well) in a 96-well plate.
2. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO<sub>2</sub>).
3. Add 10 µl of various concentrations of your toxic compound into the culture media in the respective wells.
4. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
5. Thaw the *CCVK-I* on the bench top or in a water bath at 37°C if it is frozen. It takes about 30 minutes on the bench top at 25°C or 5 minutes in the water bath at 37°C.
6. Add 10 µl of *CCVK-I* solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
7. Incubate the plate for 1-4 hours in the incubator. Measure the absorbance at 450 nm using a microplate reader. To measure the absorbance later, add 10 µl of 1% (w/v) SDS to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 48 hours.

### Background Control

1. Slight spontaneous absorbance around 460 nm occurs in culture medium incubated with *CCVK-I*. This background absorbance depends on the culture medium, pH, incubation time and length of exposure to light. Typical background absorbance after 2 hours incubation is 0.1 - 0.2 absorbance units. To correct for this, prepare one or more control wells without cells, and subtract the average absorbance of the control wells from that of the other wells.
2. During a 5-hour experiment, the absorbance of the *CCVK-I* solution does not increase at room temperature.

### Precautions

1. Since the *CCVK-I* assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity in viable cells may cause discrepancy between the actual viable cell number and the cell number determined using the *CCVK-I* assay.
2. WST-8 may react with reducing agents to generate WST-8 formazan. Please check the background O.D. if reducing agents are used in cytotoxicity assays or cell proliferation assays.
3. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. Phenol red containing culture media can be used with this kit for cell viability assays.

5. Membrane filtration is recommended for the sterilization of the *CCVK-I* solution, if necessary.
6. The incubation time varies by the type and number of cells in a well. Generally, leukocytes give weak coloration, thus a long incubation time (up to 4 hours) or a large number of cells (~10<sup>5</sup> cells/well) may be necessary.
7. Since the cytotoxicity of this kit is very low, further color development is possible after reading the absorbance.
8. Neutral red or crystal violet can be used after the *CCVK-I* assay.
9. Measure the reference wavelength at 600 nm or higher if there is a high turbidity in the cell suspension.

## Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

## FAQs

### 1. How many cells should there be in a well?

For adhesive cells, at least 1,000 cells are necessary per well (100  $\mu$ l medium) when using a standard 96-well plate. For leukocytes, at least 2,500 cells are necessary per well (100  $\mu$ l medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25,000. If 24-well or 6-well plates are used for this assay, please calculate the number of cells per well accordingly, and adjust the volume of the *CCVK-I* solution in each well to 10% of the total volume.

### 2. Can this kit be used with a 384-well plate?

Yes, you can use this kit for a 384-well plate. Add *CCVK-I* solution with 1/10 volume of the culture medium in a well. If the volume of *CCVK-I* to be added is too small, dilute *CCVK-I* in half with the medium and add 1/5 of the volume of culture medium in a well.

### 3. Does *CCVK-I* stain viable cells?

No, it does not stain viable cells because a water-soluble tetrazolium salt (WST-8) is used for the *CCVK-I* solution. The electron mediator, 1-Methoxy PMS, receives electrons from viable cells and transfers the electrons to WST-8 in the culture medium. Since its formazan dye is also highly water soluble, *CCVK-I* cannot be utilized for cell staining.

### 4. Does phenol red affect the assay?

No. The absorption value of phenol red in culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, culture medium containing phenol red can be used for the *CCVK-I* assay.

### 5. Is there a correlation between *CCVK-I* and the thymidine incorporation assay?

Yes. However, please note that since *CCVK-I* uses a different assay mechanism from that of the thymidine assay, the results may differ.

### 6. Is *CCVK-I* toxic to cells?

*CCVK-I* solution itself is cytotoxic because of the high concentration of 1-Methoxy PMS. *CCVK-I* in the culture medium, however, is not cytotoxic because of the ten times dilution with culture medium. Therefore, long incubation, such as overnight and several days, are possible. The same cell culture can be used for other cell proliferation assays, such as the crystal violet assay, neutral red assay or DNA fluorometric assays after the *CCVK-I* assay is completed. Since each cell has different tolerance to *CCVK-I*, incubate cells with *CCVK-I* solution and check the cell viability if a longer incubation time is necessary.

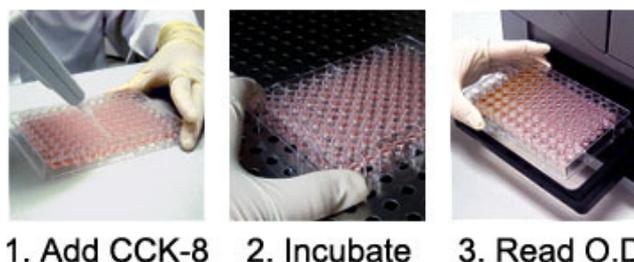
### 7. How stable is *CCVK-I*?

*CCVK-I* is stable over 6 months at room temperature and one year at 0-5°C with protection from light. For longer storage, we recommend -20°C storage condition; *CCVK-I* is stable over 2 years at -20°C.

### 8. I do not have a 450 nm filter. What other filters can I use?

You can use filters with the absorbance between 450 nm and 490 nm.

Fig. 2: Easy and convenient assays procedure using the Colorimetric Cell Viability Kit I.



1. Add CCK-8

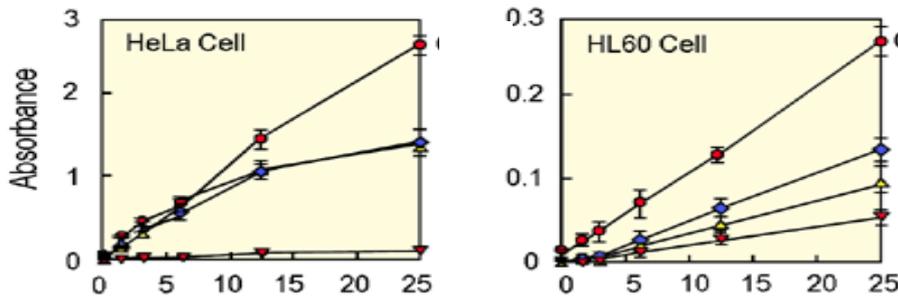
2. Incubate

3. Read O.D.

### Advantages:

- One-bottle, ready-to-use solution
- No organic solvents or isotopes required
- No harvesting, no washing and no solubilization steps
- More sensitive than MTT, XTT, or MTS

Fig. 3: Cell proliferation assay using CCVK-I and other reagents (number of cells: 1000/well)



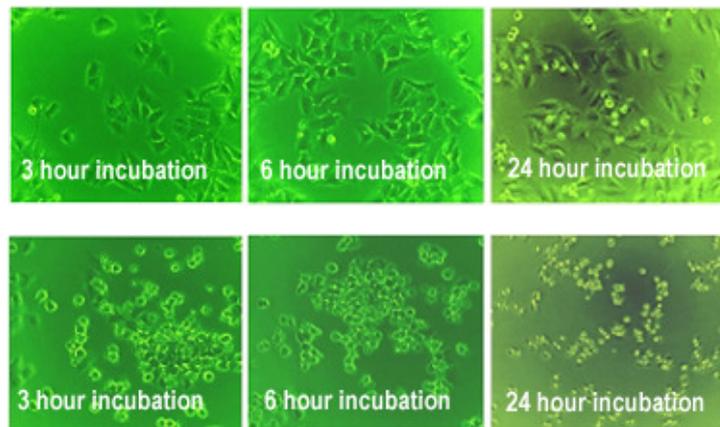
**Detection Methods:**

- CCVK-I ●: 450 nm, reference: 650 nm
- XTT ◆: 450 nm, reference: 650 nm
- MTS ▲: 490 nm, reference: 650 nm
- MTT ▼: 570 nm, reference: 650 nm

**Culture medium:**

- MEM, 10% FCS, L-glutamine (HeLa)
- RPMI1640, 10% FCS, L-glutamine (HL60)
- Staining:** 37°C, 5% CO<sub>2</sub>, 2 h (HeLa)
- 37°C, 5% CO<sub>2</sub>, 3 h (HL60)

Fig. 4: Toxicity of assay solutions: Comparison between CCVK-I and other assay kits.



Top: PromoKine's Colorimetric Cell Viability Kit I (WST-8)

Bottom: Cell Counting Kit from a competitor.

Procedures and Conditions: Preincubate HeLa cell/DMEM culture overnight in a CO<sub>2</sub> incubator, and add 10 µl of CCVK-I solution to each well. Incubate cells at 37°C for various time periods, and observe the cells with a phase-contrast microscope.

As seen in the photos, even after 24-hour incubation with CCVK-I, cells continue to proliferate. Other cell viability assay kits are toxic to cells, and most of the cells are killed during the first 3 hours of incubation.

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