

■ General Information

Cellrix® Viability assay kit allows very convenient assays by utilizing the highly water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium,monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron carrier. WST-8 is reduced by dehydrogenases in cells to give a yellow colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells.

Cellrix® Viability assay kit, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. The detection sensitivity of **Cellrix® Viability assay kit** is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Cellrix® Viability assay kit is a ready-to-use one-bottle solution which offers a simple, rapid, reliable measurement of cell viability and the cytotoxicity of agents within various chemicals quantitatively.

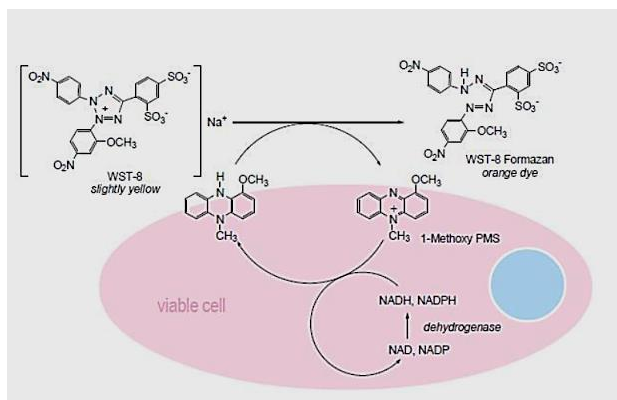


Fig. 1. Principle of the cell viability detection with Cellrix® Viability assay kit

■ Kit Contents

Component	Assay	Catalog No.
5ml bottle X 1	500 tests	B1007-500
5ml bottle X 3	1500 tests	B1007-1500
5ml bottle X 5	2500 tests	B1007-2500



■ Storage

Cellrix® Viability assay kit is stable over one year at 0-5°C with protection from light. Store it at -20°C for longer storage. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. Please store the kit at 0-5°C for frequent use.

■ General Protocol

◆ Cell Number Determination

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO₂).
2. Add 10 µl of the **Cellrix® Viability assay kit** solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
3. Incubate the plate for 1 - 4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.

◆ Cell Proliferation and Cytotoxicity Assay

1. Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO₂).
2. Add 10 µl of various concentrations of substances to be tested to the plate.
3. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
4. Add 10 µl of **Cellrix® Viability assay kit** solution to each well of the plate.
Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
5. Incubate the plate for 1 - 4 hours in the incubator.
6. Measure the absorbance at 450 nm using a microplate reader.

■ Precautions

1. Slight spontaneous absorbance around 450 nm occurs in culture medium incubated with **Cellrix® Viability assay kit**. 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. We recommend that number of cells and optimal reaction time is determined from preliminary experiment because the incubation time varies by the type and number of cells in a well.
3. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. 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.