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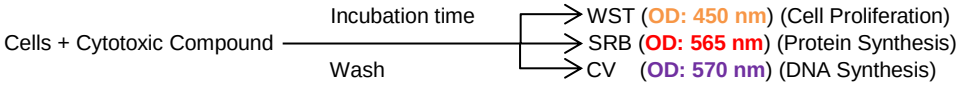
Cell Cytotoxicity Assay Kit II (WST-SRB-Crystal Violet)

10/18

(Catalog # K173-1000; 1000 assays, Store kit at -20°C)

I. Introduction:

Cytotoxicity assays are essential tools in drug discovery. *In vitro* single parameter cytotoxicity assays, such as using tetrazolium salt (WST), sulforhodamine B (SRB) or crystal violet (CV) are widely used in cultured cellsin order to assess the cytotoxicity of potential drug candidates as these methods are fast, reproducible, and do no require using living mammals during early drug discovery stages. However, the most critical problem of using single parameter assays is the determination of their IC_{50} data offers a limited perspective and can lead to erroneous assumptions or erroneous, unsucessful results when the selected candidates are used in more complex models (false positives). On the other hand, if the cytotoxicity assay is not selected appropriately, false negative compounds that are actually cytotoxic may be easily missed. Thus, the analysis of candidates as potential cytotoxic compounds is usually more comprehensive when two or more cytotoxicity assays are performed. Additionally, SRB, WST and CV cyototoxic assays offer relatively inexpensive, accurate, yet reliable results in a timely manner. BioVision's WST-SRB-Crystal Violet Combined Cytotoxicity Assay Kit allow researchers to test the cytotoxicity effect of their candidate compounds in different biological processes: cell proliferation (WST), protein synthesis (SRB) and/or total DNA synthesis (CV). The assays are compatible to each other and same set of cultured cells can be tested using these assays. The kit is simple, fast, high-throughput compatible and provides an efficient research tool for the quantitative measurement of cell cytotoxicity in terms of viability, protein and DNA synthesis.



II. Application:

- *In vitro* cell proliferation, cytotoxicity studies
- High-throughput drug screening

III. Sample Type:

- Cell culture: Adherent cells and non-adherent cells

IV. Kit Contents:

Components	K173-1000	Cap Code	Part Number
WST Reagent (lyophilized)	2 vials	Green	K173-1000-1
WST Developer	2 x 5 ml	Amber	K173-1000-2
SRB Solution	50 ml	Amber	K173-1000-3
20X SRB Wash	50 ml	WM	K173-1000-4
10X SRB Developer	22 ml	WM	K173-1000-5
CV Solution	40 ml	WM	K173-1000-6
CV Developer	100 ml	WM	K173-1000-7
Fixation Solution	55 ml	NM	K173-1000-8
20 mM Doxorubicin	100 µl	Red	K173-1000-9

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- 100% methanol

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. WST, SRB and CV dyes are light-sensitive and should be protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Bring all reagents to room temperature before use.

- **WST Reagent and WST Developer**: Dissolve WST Reagent (1 vial) by using 5 ml of WST Developer to make WST solution. Aliquot WST solution (*1 ml is sufficient for one 96 well plate assays*) and store at -20°C. WST solution is stable for 1 year at -20°C. Protect from light. Avoid repeated freeze-thaw.
- **Fixation Solution, SRB Solution, 20X SRB Wash and 10X SRB Developer**: Fixation and SRB Solutions are ready to use. *For one 96-well plate,* mix 5 ml of 20X SRB Wash with 95 ml of dH_2O to prepare 1X SRB Wash. Mix 2 ml of 10X SRB Developer with 18 ml of water to prepare 1X SRB Developer. After use, store SRB Solution at -20°C and other reagents at 4°C.
- **CV Solution and CV Developer**: Bring CV Solution to room temperature before use. Add 11 ml of 100% methanol (not supplied) into the bottle. Shake contents and let it stand for 15 minutes at room temperature. *One 96 well plate requires 5 ml of CV Solution*. CV Developer is ready to use. After use, store the CV Solution at -20°C and the CV Developer at 4°C.
- **20 mM Doxorubicin**: Ready to use. Store at -20°C.

VII. WST-Sulforhodamine-Crystal Violet Combined Cytotoxicity Assay Protocol:

This assay was developed with MCF7 cells (adherent). However, any adherent of suspension cell line can be used. The protocol below refers to a 96-well tissue culture plate; adjust volumes accordingly for other plate formats. Growth conditions, cell number per well and

other factors may affect cell growth, proliferation and cytotoxicity; therefore optimize the assay for your cell type. Avoid stressing the cells by excessive washes or temperature changes prior to performing the assays. All steps should be carried out at room temperature (RT) unless otherwise specified; all buffers brought to RT prior to the experiment.

NOTE: you may use the same set(s) of cultured cells to perform WST, SRB and Crystal Violet assays sequentially. Do not alter the order of these assays.

The correct sequence of assays to be performed is: 1. WST; 2. SRB; 3. Crystal Violet.

1. **Cell Culture**: Grow cells to ~80% confluency. ***For adherent cells***: trypsinize the cells and spin down at RT and 2000 x g for 5 min the cells. ***For suspension cells***: spin down cells at RT and 2000 x g for 5 min before removing any solution (**use the same conditions for all medium exchanges, detection steps and washes**).

   For both types of cells: Remove the solution and add fresh culture medium to disperse the cell pellet. Determine the cell density by using a hemocytometer or cell counter. Adjust the cell concentration if necessary to 50000 – 250000 cells/ml. Add 100 µl of the cells typically containing between 5,000–25,000 cells/well to a 96-well clear flat-bottom plate. Incubate cells overnight at 37 °C, 5% CO_2 controlled incubator

2. **Compound Treatment:** Prepare compounds using DMSO as solvent. *Recommended final DMSO concentration in wells should be 0.5% or less*. Add diluted compounds to the wells. Prepare a DMSO vehicle control and a background control (culture media only). For inhibitor control, add 1 µl of 20 mM doxorubicin to a well containing the cells. Incubate the plate for 72 hrs at 5% CO_2 and 37 ºC.

3. **WST Detection**: After compound incubation, spin as above, aspirate culture medium. Add 100 µl of fresh, warm culture medium to the wells. Add 10 µl of WST solution into each well. Incubate the cells in the incubator for 1-3 hrs. Tap the plate gently and do not disturb the cell monolayer. Measure the OD 450 nm.

   Note: An orange color in vehicle control wells is an indication of proper cell density (5000-25000 cells/wells). Pale coloration may indicate low cell densities, and longer incubation times are required.

4. **SRB Detection**: After measurement at 450 nm, spin as above, aspirate the wells and add 100 µl of culture medium to the wells. Add 25 µl of Fixation Solution to each well. Incubate the plate for 1 hr at 4 ˚C. Spin and remove the solution and use 200 µl of dH_2O to wash the wells once. *Washing should be done as gentle as possible to avoid disturbance of the cell monolayer*. Aspirate wash(es) as much as possible by pipetting. Add 45 µl of SRB Solution to each well and stain for 15 min at room temperature in the dark. *Protect plates from light*. After staining, aspirate the wells and use 200 µl of 1X SRB Wash to wash each well. Repeat wash 4 times. At the end of washing, aspirate the wells by pipetting. Add 100 µl of 1X SRB Developer into each well. Incubate the plate at room temperature for 10 min and tap it occasionally. Measure the OD 565 nm.

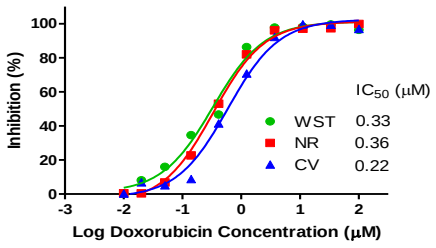
5. **CV Detection:** After measurement at 565 nm, aspirate the wells as much as possible. Add 50 µl of CV Solution into each well. Incubate the plate at room temperature for 15 min. Use 250 µl of dH_2O to wash the cells and repeat washing for 4 times. Aspirate the wells by pipetting. Add 100 µl of CV Developer into each well. Shake the plate in a shaker at room temperature for 15 min. Measure the OD 570 nm.

6. **Calculations:** Correct the background by subtracting the O.D. of the background control from all readings. Calculate the percentage of inhibition using the formula below:

$$\% \text{ Inhibition} = \frac{\text{O.D.}_{\text{VEHICLE}} - \text{O.D.}_{\text{sample}}}{\text{O.D.}_{\text{VEHICLE}}} \times 100\%$$

Where: $\text{O.D.}_{\text{DMSO}}$ is the O.D. of the DMSO control after background correction
$\text{O.D.}_{\text{sample}}$ is the O.D. of the sample after background correction.

A.



B.

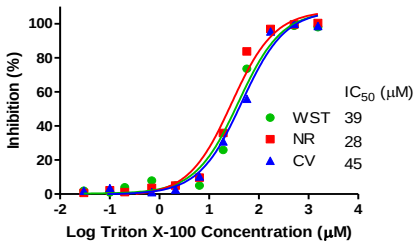


Figure A. Dose-response curves of Doxorubicin detected by the assay kit in the MCF-7 cells. **B**. Dose-response curves of Triton X-100 detected by the assay kit in the MCF-7 cells.

VIII. RELATED PRODUCTS:

WST-NR-CV Combined Cytotoxicity Assay Kit (K543)
Neutral Red Cell Cytotoxicity Assay Kit (K447)
MTT Cell Proliferation Assay Kit (Colorimetric) (K299)
MTS Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric) (K943)
Crystal Violet Cell Cytotoxicity Assay Kit (K329)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)

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