

## ab284555– WST-NR-CV Combined Cytotoxicity Assay Kit

For the quantitative measurement of cytotoxicity in viable cells.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284555>

### Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

### Materials Supplied

Item	Quantity	Storage Condition
WST Reagent (lyophilized)	2 vials	-20°C
WST Developing Solution	2 × 5 mL	-20°C
100X NR Staining Solution	2 mL	-20°C
2X NR Solubilization Solution	25 mL	-20°C
CV Staining Solution	40 mL	-20°C
10X CV Solubilization Solution	10 mL	-20°C
10X Washing Solution	115 mL	-20°C
20 mM Doxorubicin	100 µL	-20°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

### Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

**WST Reagent and WST Developing Solution:** Dissolve lyophilized WST reagent (1 vial) by using 5 ml of WST Developing Solution 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.

**100X NR Staining Solution:** Prepare 1X NR Staining Solution. For one 96 well plate, dilute 0.2 ml of 100X NR Staining Solution by 100 folds to 20 ml by using the culture medium. Note: Do not store 1X NR Staining Solution. Discard unused Staining Solution if not used within 24 hours.

**2X NR Solubilization Solution:** Prepare 1X NR Solubilization Solution. For one 96 well plate, mix 7.5 ml of 2X NR Solubilization Solution with 7.5 ml of 100% methanol (not supplied) to make 1X NR Solubilization Solution. 1X NR Solubilization Solution is stable and can be stored at 4°C for 3 months.

**CV Staining Solution:** Bring it to room temperature before use. Add 11 ml of 100% methanol into the bottle. Shake contents and let it stand for 15 minutes at room temperature. 5 ml of CV Staining Solution is required for one 96 well plate. After use, store it at -20°C.

**10X CV Solubilization Solution:** Prepare 1X CV Solubilization Solution. For one 96 well plate, mix 1 ml of 10X CV Solubilization Solution with 9 ml of water. After use, store it at room temperature.

**10X Washing Solution:** Prepare 1X Washing Solution by adding 1 part 10X Washing Solution to 9 parts dH<sub>2</sub>O. Store at 4°C.

**20 mM Doxorubicin:** Ready to use. Store at -20°C.

### Assay Protocol

#### Screening Compounds, Inhibitor Control & Background Control preparations:

#### Cell Culture:

1. Grow cells to ~80% confluency. **For adherent cells**, trypsinize the cells and spin down the cells. **For suspension cells**, spin down cells before removing any solution (same for detection below).
2. Remove the solution and add culture medium to disperse the cell pellet.
3. Determine the cell density by using a hemocytometer.
4. Adjust the cell concentration if necessary, to 50000 - 250000 cells/ml.
5. Add 100 µl of the cells typically containing between 5,000–25,000 cells/well to a 96-well clear flat-bottom plate.
6. Incubate cells overnight at 37 °C, 5% CO<sub>2</sub> controlled incubator.

#### Compound Treatment:

1. Prepare compounds using DMSO as solvent. Dilute Stock solutions in DMSO appropriately. Recommended final DMSO concentration in wells should be 0.5% or less.
2. Add diluted compounds to the wells.
3. Prepare a DMSO vehicle control and a background control (containing only the medium). **For inhibitor control**, add 1 µl of 20 mM doxorubicin to a well containing the cells.
4. Incubate the plate for 72 hrs at 5% CO<sub>2</sub> and 37 °C.

#### WST Detection:

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

#### NR Detection:

1. After compound incubation, aspirate culture medium.
2. Add 200 µl of 1X Washing Solution to wash cells once. Washing must be done as gentle as possible to avoid disturbance of the cell monolayer.
3. Remove wash solution as much as possible by pipetting. Add 150 µl of 1X NR Staining Solution to each well and stain for 2 hrs. in an incubator.
4. After incubation, remove the solution by pipetting. Add 200 µl of 1X Washing Solution to wash the cells. Repeat twice.
5. Remove wash solution as gentle as possible. Fixation (eg. by using 4% paraformaldehyde, not included) sometimes may be required for low adherent cells.
6. Add 150 µl of 1X NR Solubilization Solution to the wells. Incubate the cells in the controlled incubator for 20 min.
7. Tap the plate gently and avoid disturbing cell monolayers. Measure the OD at 540 nm.

**CV Detection:**

1. After compound incubation, aspirate culture medium.
2. Add 200 µl of 1X Washing Solution to wash cells once.
3. Remove the solution as gentle as possible.
4. Add 50 µl of CV Staining Solution into each well. Incubate the plate at room temperature for 15 min.
5. Use 250 µl of dH<sub>2</sub>O to wash the cells for 4 times.
6. Add 100 µl of 1X CV Solubilization Solution into each well.
7. Shake the plate in a shaker at room temperature for 20 min.
8. Measure the OD at 570 nm.

**Calculation**

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

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

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