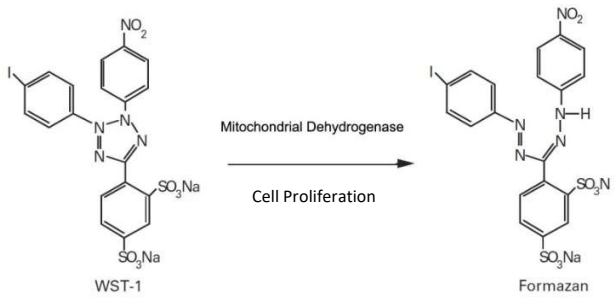
WST-1 Cell Proliferation Reagent (BN00562)

(Catalog BN00562; 2500 assays; Store at -20°C)

I. Introduction:

The ready-to-use cell proliferation reagent, WST-1 provides a simple and accurate method to measure cell proliferation, which is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion in the number of viable cells results in an increase in the activity of the mitochondrial dehydrogenases, which in turn leads to increase in the amount of formazan dye formed. The formazan dye produced by viable cells can be quantified by measuring the absorbance at 440 nm. This new method is non-radioactive, rapid and more sensitive than MTT, XTT, or MTS-based assays. The entire assay can be performed in the same microtiter plate and does not require extra steps like washing, harvesting and cell solubilization.



II. Application:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc.
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Assessment of physiological mediators and antibodies that inhibit cell growth.

III. Sample Type:

Adherent or suspension cells cultured in 96-well microtiter plate.

IV. Kit Contents:

Components	2500 assays
WST-1 Reagent (in electron coupling solution)	25 ml

V. User Supplied Reagents and Equipments:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Aliquot the WST-1 Reagent and store at -20°C. The WST-1 Reagent is stable for a few weeks at 4°C, and 6 months at –20°C. It is recommended to prepare aliquots of the solution (1 ml is sufficient for assay with one 96-well microtiter plate), to avoid freeze/thaw.

VII. Cell Proliferation Assay Procedure:

1. Culture cells (0.1 – 5 x 10^4/well) in a 96-well microtiter plate in a final volume of 100 µl/well culture medium in the absence or presence of various amounts of the factors tested. (**Note:** For toxicity assays, use more cells to start with (e.g., 5 x 10^4 – 5 x 10^5 cells/well.)
2. Incubate cells for 24 - 96 hrs.
3. Add 10 µl WST-1 Reagent to each well. (**Note:** If the cells are cultured in different volume of culture medium, increase or decrease the amount of WST-1 Reagent correspondingly.)
4. Incubate the cells for 0.5 – 4 hrs in standard culture conditions. (**Note:** The appropriate incubation time depends on the individual cell type and cell concentration used. Therefore, it is recommended to determine the optimal incubation time for the particular experimental setup used.)
5. Shake for 1 min on a shaker to mix the contents.
6. Measure the absorbance of the treated and untreated samples using a microtiter plate reader at 420 - 480 nm according to the filters available for the plate reader. The reference wavelength should be ~ 650 nm.

Notes:

- Use the same amount of culture medium and WST-1 Reagent in an empty well as a blank control for the microtiter plate reader.
- The assay can be stopped by adding 10 µl of 1% SDS into each well, and gentle mixing.
- Phenol Red in culture medium does not significantly interfere with the reading.

FOR RESEARCH USE ONLY! Not to be used on humans.