Quick Cell Proliferation Colorimetric Assay Kit

(Catalog #K301-500, -2500; Store at –20° C)

I. Introduction:

The Quick Cell Proliferation Assay Kit provides all reagents and detailed instructions for a fast and sensitive quantification of cell proliferation and viability. The assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion in the number of viable cells resulted in an increase in the activity of the mitochondrial dehydrogenases, which leads to the increase in the amount of formazan dye formed. The formazan dye produced by viable cells can be quantified by multi-well spectrophotometer (microtiter plate reader) by measuring the absorbance of the dye solution at 440 nm. The assay can be used for the measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc. It can also be used for the analysis of cytotoxic compounds like anticancer drugs and many other toxic agents and pharmaceutical compounds. The new method is so simple, requiring no washing, no harvesting, and no solubilization steps, and is faster and more sensitive than MTT, XTT, or MTS-based assays. The entire assay can be performed in a microtiter plate.

II. Kit Contents:

Component	K301-500 500 assays	K301-2500 2500 assays	Part Number
WST-1 Reagent (lyophilized)	1 vial	1 vial	K301-xxx(x)-1
Electrocoupling Solution (ECS)	5 ml	25 ml	K301-xxx(x)-2

III. Reagent Preparation and Storage:

Dissolve 1 vial of lyophilized WST-1 reagent with 5 ml (or 25 ml) of the Electro Coupling Solution (ECS), aliquot the WST-1/ECS solution and store at -20° C. The WST-1/ECS Solution is stable for 3 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).

IV. Cell Proliferation Assay Procedures:

1. Culture cells (0.1 - 5x10^4/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., 5x10^4 – 5x10^5 cells/well).
2. Incubate cells for 24-96 hours.
3. Add 10 µl/well WST-1/ECS solution to each well.
   Note: If the cells are cultured in different volume of culture medium, increase or decrease the amount of WST-1/ECS solution correspondingly.
4. Incubate the cells for 0.5 – 4 hours 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 thoroughly for 1 min on a shaker.
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 ~ 650nm.

Notes:

1. Using the same amount of culture medium and WST-1/ECS solution in an empty well as a blank position for the microtiter plate reader.
2. The assay can be stopped by adding 10 µl of 1% SDS into each well, and shake mix.
3. Phenol Red in culture medium does not significantly interfere with the reading.

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- Mammalian Cell Extraction Kit
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