MBL International Corporation

Quick Cell Proliferation Assay Kit

Catalog No.: BV-K301-10 (500 assays); BV-K301-2500 (2500 assays)
(Store kit at –20°C)

I. Introduction:

The Quick Cell Proliferation Assay Kit provides all reagents and detailed instructions for a fast 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 overall activity of the mitochondrial dehydrogenases in the sample. The augmentation in enzyme activity leads to increase in the amount of formazan dye formed. The formazan dye produced by viable cells can be quantified by multiwell spectrophotometer (microtiter plate reader) by measuring the absorbance of the dye solution at 440 nm. The assay can be used for the measurement of the proliferation in response to growth factors, cytokines, mitogens, and nutrients. It also can 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-based assays. The entire assay can be performed in one microtiter plate.

II. Kit Contents:

Components	BV-K301-10	BV-K301-2500
	500 assays	2500 assays
WST-1 Reagent (lyophilized) Electro Coupling Solution (ECS)	1 vial 5 ml	1 vial 25 ml

Reagent Preparation: Dissolve the 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.

III. Cell proliferation Assay Procedures:

1. Culture cells (0.1-5 x 10^4/wells) in a 96-wells microtiter plate in a final volume of 100µl/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/wells.)
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 using to the filters available for the plate reader. The reference wavelength should be more than 600 nm.

   Note: Using the same amount of culture medium and WET-1/ECS solution in an empty as a blank position for the microtiter plate reader.

IV. Storage and Stability:

- Store kit at –20°C. Protect from light.
- The WST-1/ECS Solution is stable for 3 month at -20°C. It is recommended to prepare aliquots of the solution (1 ml is sufficient for assay with one 96-well microtiter plate)

V. References:

Decker, T. and Lohmann-Matthes, M.L. (1988) *J. Immunol Methods* **115**: 62-69.

Korzeniewski, C. and Callewaert, D.M. (1983) *J. Immunol Methods* **64**: 313-320.