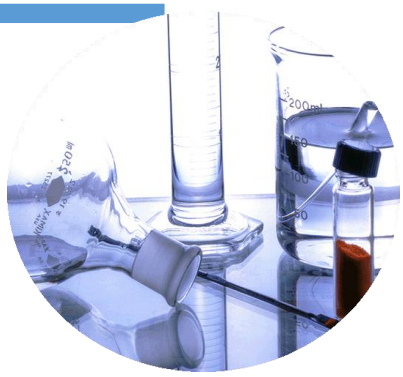
WST-8 Cell Proliferation Kit

INSTRUCTION MANUAL

Assays kits



WST-8 Cell Proliferation Kit

The WST-8 Cell Proliferation Kit is a ready-to-use assay kit for determination of viable cell number and for studying induction or inhibition of cell proliferation *in vitro*.

	Content	Catalog Number	Number of assays (96-well plate)
WS1000	10 mL of WST-8 Solution	WS1000	**1000**

For any technical questions, contact us at tech@ozbiosciences.com

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1. Technology

1.1. Description

The WST-8 Cell Proliferation Kit is a colorimetric assay for the determination of viable cell number and for studying induction or inhibition of cell proliferation *in vitro*. This assay kit is based on the cellular reduction of the tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] into a highly water-soluble, orange-colored formazan dye upon reduction in the presence of an electron carrier. As opposed to MTT assay, no solubilisation process is required since this formazan does not require solvation: the WST-8 is soluble in the tissue culture medium.

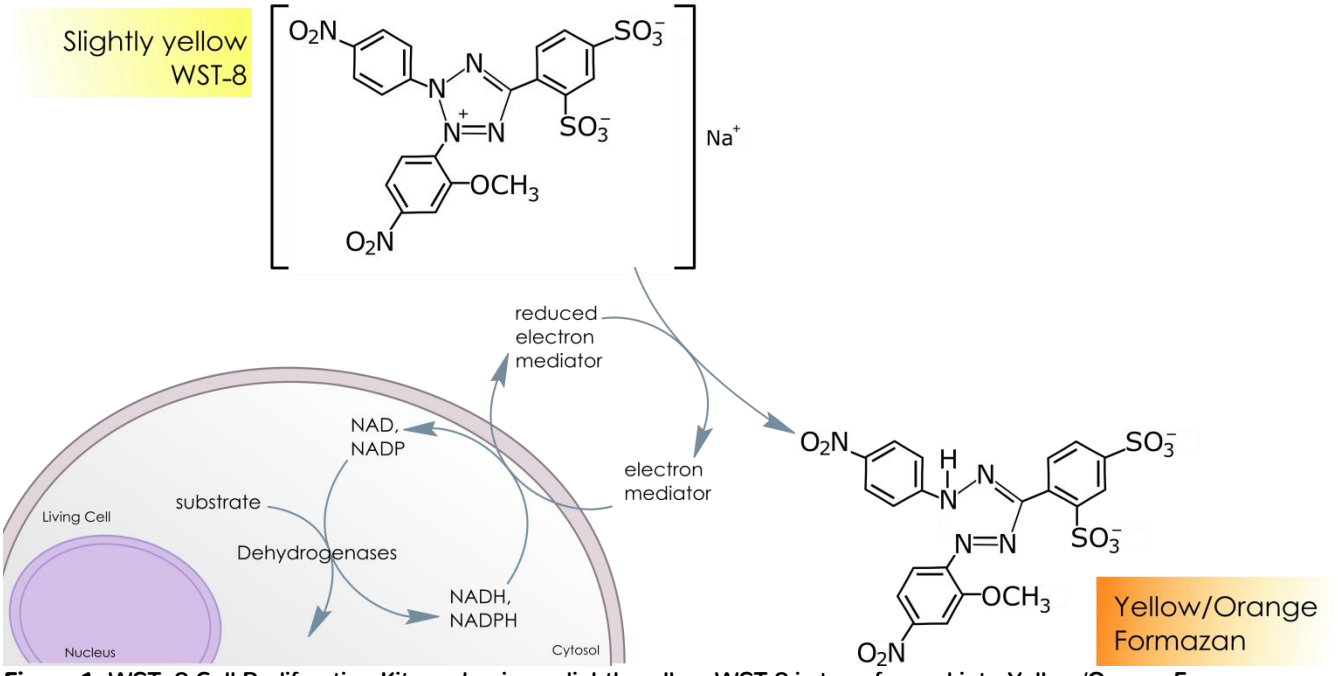

Figure 1: WST- 8 Cell Proliferation Kit mechanism: slightly yellow WST-8 is transformed into Yellow/Orange Formazan.

The WST-8 Cell Proliferation Kit is more sensitive at neutral pH than other tetrazolium salts such as MTT, XTT, MTS or WST-1 and the amount of formazan produced is directly proportional to the number of living cells.

Colorimetric measurement at 450 nm allows quantification of viable cells. The WST-8 Cell Proliferation Kit does not need cell fixation, cell lysis or washing steps rendering this ready-to-use kit fast, accurate, sensitive and adapted to high throughput screening.

1.2. Storage and shipping condition

Storage: Upon reception, store the WST-8 Cell Proliferation Kit at 4°C. Protect from light.
Stability: 1 year at 4°C

Shipping condition: The kit is shipped at RT.

2. Applications and Protocols

2.1. General Considerations

- Avoid repeated thawing and freezing cycles that could increase the background signal.
- This kit is compatible with phenol red containing medium.
- Incubation time varies with the type and number of cells; long incubation time (up to 4 hours) can be used for an optimal measurement.
- Allow reagent to reach room temperature before starting. Avoid direct exposure to- and protect from light.

2.2. General protocol for 96-well plate

Cell preparation:

1. Seed cells in a 96-well plate under standard culture conditions.
2. Carry out experiment by adding chemical compounds or biological agents to cells

Measuring cell proliferation or viability:

3. Add 10 µL of WST-8 solution.
4. Incubate cells 30 min to 4h at 37°C in the dark (incubation time varies depending on the metabolic activity of the cells).
5. Optionally: centrifuge cells and apply protocol on the supernatant (see below)
6. Measure absorbance at 450 nm.
7. Subtract background absorbance of the non-treated cells from all other values.

Optionally for suspension cells, a centrifugation procedure to pellet cells (5 min x 1200 rpm) would allow working on supernatant to lower background signal.

2.3. General protocol for other well format

This kit is compatible with any cell culture format, from 384-well to 6-well plates. Follow the same protocol than for 96-well plates; changes only apply on the volumes used:

- Step 3: adjust the volume of the WST-8 solution in a well to 10% of the total volume.

2.4. Performance characteristics

Cell titration and viability measurement after transfection.

This kit is compatible with both cell suspension and adherent cells. Cell titration was performed using JurKat T cell line cultivated in suspension and viability was measured using HEK-293 adherent cell line.

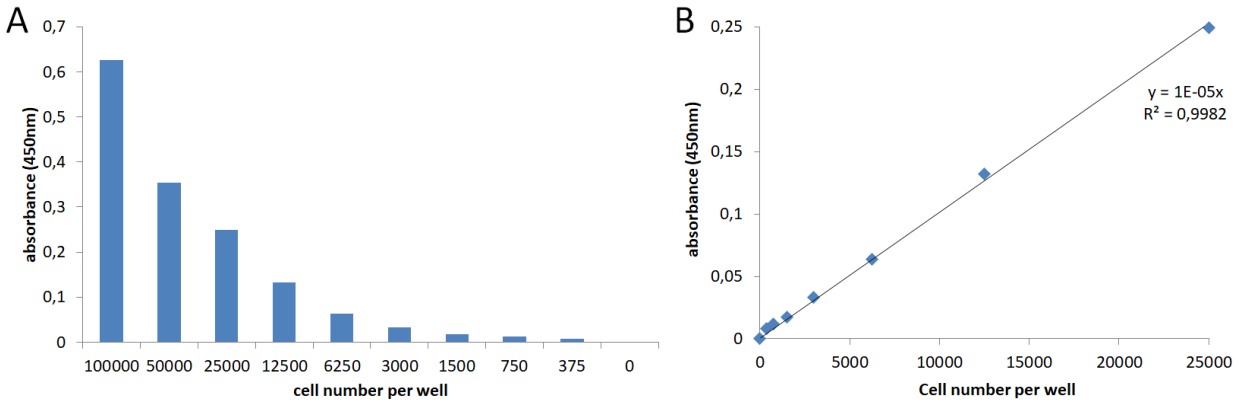

Figure 2: Cell number titration. (A) Jurkat T cells were seeded at different concentrations in a 96-well plate and incubated 2h with WST-8 Cell Proliferation Kit. Linearity between absorbance and cell number varies according to the cell type and metabolism (B).

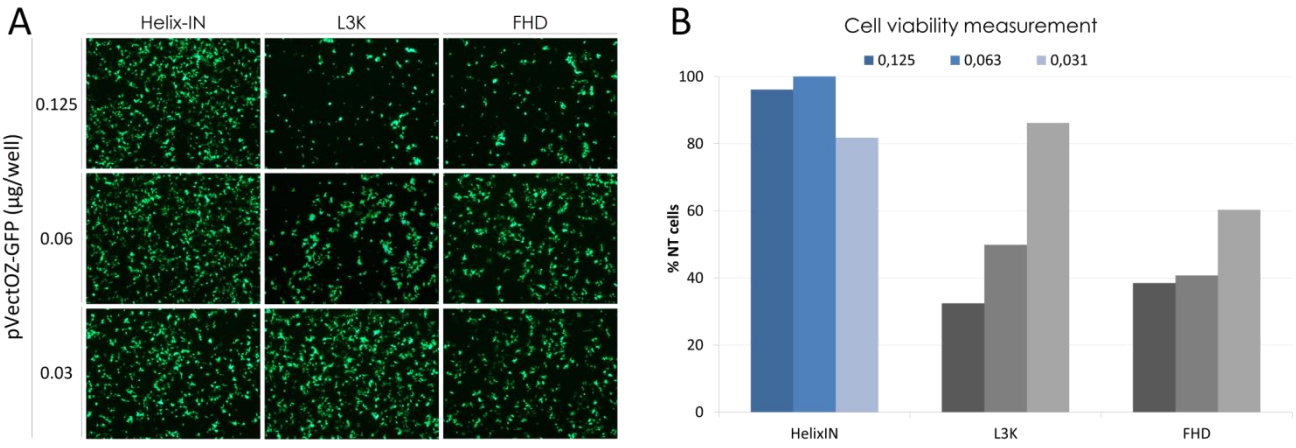

Figure 3: Cell viability measurement after transfection. (A) HEK-293 cells were cultivated in 96-well plates and transfected with 0.03, 0.06 and 0.125 µg DNA per well using Helix-IN DNA Transfection Reagent (OZ Biosciences) at a 2:1 ratio and two other transfection reagents according to their respective manufacturer's protocols. After 48h, transfection efficiency was monitored by fluorescence microscopy (B) and viability was determined as a % of Non-transfected (NT) cells using WST-8 Cell Proliferation Kit. 10µL of WST-8 Reagent were added to the cells and Absorbance at 450 nm was measured after 2h incubation at 37°C.

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