**Product Information Sheet** 

# WST-8

**Water-Soluble Tetrazolium 8** 

# **Catalogue Number SBR00126**

# **Product Description**

WST-8 is a water-soluble tetrazolium salt commonly used in colorimetric assays to measure cell viability and enzyme activity. Upon reduction, primarily by mitochondrial dehydrogenases in viable cells, it produces a yellow-orange formazan dye, which can be quantitatively measured via absorbance, reflecting the metabolic activity of the cells.

To enhance electron transfer and boost sensitivity, 1-methoxy-5-methylphenazinium methyl sulfate (1-methoxy PMS or mPMS) is employed as an intermediate electron carrier (see Figure 1).

#### Figure 1.

Cell viability detection mechanism of WST-8 with 1-Methoxy PMS. This combination forms the foundation of the WST-8/mPMS assay, a widely used method due to its simplicity, high sensitivity, and non-radioactive nature. The WST-8/mPMS system is advantageous over traditional MTT assays because the WST-8 formazan product is water-soluble, eliminating the need for organic solvents in solubilization steps.



# **Applications:**

- Cell Viability and Cytotoxicity Assays:
  Used extensively in drug screening,
  toxicology, and cancer biology to
  determine cell proliferation or cytotoxic
  effects. The pairing with mPMS ensures
  better electron transfer and reduces
  variability across assay formats.<sup>2-3</sup>
- Dehydrogenase Enzyme Assays: The WST-8/mPMS system efficiently monitors NAD(P)H production, making it ideal for studying enzymes such as lactate dehydrogenase and other oxidoreductases.<sup>4</sup>
- Microbial Viability: Rapid detection of live microorganisms through metabolic activity was demonstrated using the WST-8-mPMS assay in biosensing platforms.<sup>5</sup>
- Comparative Studies with Other Tetrazolium Salts: Studies have shown that WST-8, when paired with mPMS, outperforms or complements other tetrazolium-based systems (such as MTT, XTT) in terms of accuracy and biocompatibility in 2D and 3D cultures.<sup>6</sup>

# Reagents and Equipment Required but Not Provided

- Ultrapure water
- 96 well flat-bottom plate
- Fluorescence multiwell plate reader
- 37 °C + 5% CO<sub>2</sub> incubator
- Multichannel pipettes (10 and 100 μl).
- 1-Methoxy PMS (Catalogue# M8640)
- Sodium Chloride (Catalogue# S9625)

#### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

# Storage/Stability

Store at 4 °C and protect from light.

## **Preparation Instructions**

WST-8 Working Solution Preparation for Cell Viability and Enzymatic Detection Assays:

Prepare WST-8 solution in an aqueous solution containing 5 mM WST-8, 0.2 mM 1-Methoxy PMS, and 150 mM NaCl buffer.

#### Example Preparation (5 mL Solution)

- Prepare a stock solution of 150 mM NaCl by dissolving 175.3mg in 20 mL purified water.
- Prepare a stock solution of 2 mM (0.68 mg/mL or 3.4 mg/5 mL) of 1-methoxy
   PMS in 150 mM NaCl.
- Dissolve 15 mg of WST-8 in 4ml 150 mM NaCl, then add 500µL 2mM 1-methoxy PMS. Mix well and then bring to a final volume of 5 mL by adding 500µL of 150mM NaCl.

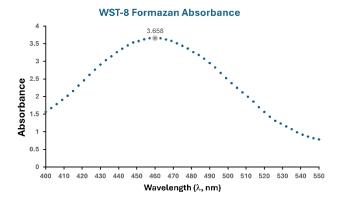
# Recommended Storage of Solution

Store the solution at 4 °C. Under these conditions, the solution is stable for up to 2 years.

# Procedure for Cell Viability Assay

1. Grow cell suspension in a 96-well microplate (100  $\mu$ l) at 37 °C in a humidified incubator with 5% CO<sub>2</sub> for the required time.

- 2. Add 10  $\mu$ l of the WST-8 solution to the cells (1:10 dilution).
- 3. Incubate for 0.5-4 hours at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.
- Measure the absorbance using a microplate reader at 420 to 480 nm (maximum absorption at 460 nm, see figure 2, WST-8 formazan absorbance).



# Figure 2.

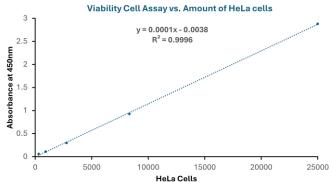
Well plate max wavelength ( $\lambda$ ) absorbance of WST-8 formazan analysis. The graph above illustrates the maximum absorbance peak of WST-8 solution ( $\lambda_{max} = 460$ nm) after incubation (2h) with viable cells, indicating the conversion of WST-8 into soluble formazan dye.

## Results

Based on the procedure above, WST-8 solution was tested on HeLa cells (0.3-2.5 x  $10^4$ ) after 2 hours of incubation at 37 °C in a humidified incubator with 5% CO<sub>2</sub>.

**Note**: Microplate reading performed at 450nm absorbance as described in most protocols. (see Figure 3)

**Figure 3.** WST-8 working solution cell viability vs. number of HeLa cells analysis.



#### References

- Tominaga, H., et al., A water-soluble tetrazolium salt useful for colorimetric cell viability assay. Analytical Communications, 36(2), 47-50. (1999)
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- Lutter, A. H., et al., Applying XTT, WST-1, and WST-8 to human chondrocytes: A comparison of membrane-impermeable tetrazolium salts in 2D and 3D cultures. Clinical hemorheology and microcirculation, 67(3-4), 327-342. (2017)

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