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.[1] 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.[2]

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).

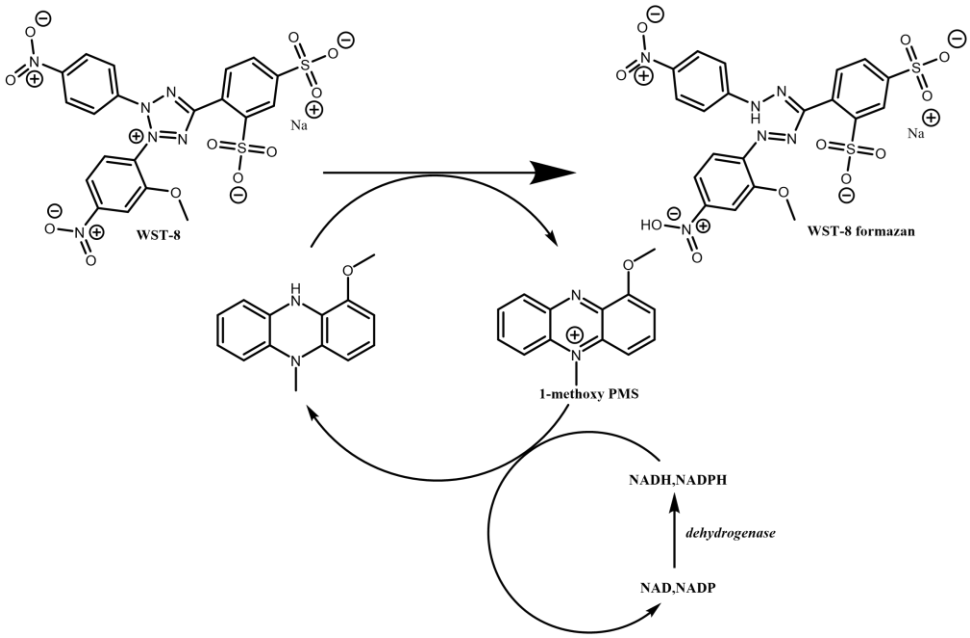

Figure 1.
Cell viability detection mechanism of WST-8 with 1-Methoxy PMS. This combination forms the foundation of the WST-8/mPMS assay, a widely used method due to its simplicity, high sensitivity, and non-radioactive nature.[1-2] The WST-8/mPMS system is advantageous over traditional MTT assays because 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.[2-3]
- **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.[4]
- **Microbial Viability**: Rapid detection of live microorganisms through metabolic activity was demonstrated using the WST-8-mPMS assay in biosensing platforms.[5]
- **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.[6]

Reagents and Equipment Required but Not Provided

- Ultrapure water
- 96 well flat-bottom plate
- Fluorescence multiwell plate reader
- 37 °C + 5% CO_2 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 -20 °C and protect from light.

Preparation Instructions

WST-1 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)

1. Prepare a stock solution of 150 mM NaCl by dissolving 175.3mg in 20 mL purified water.
2. 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.
3. 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 µl) at 37 °C in a humidified incubator with 5% CO_2 for the required time.
2. Add 10 µl 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_2.

4. Measure the absorbance using a microplate reader at 420 to 480 nm (maximum absorption at 460 nm, see figure 2, WST-8 formazan absorbance).

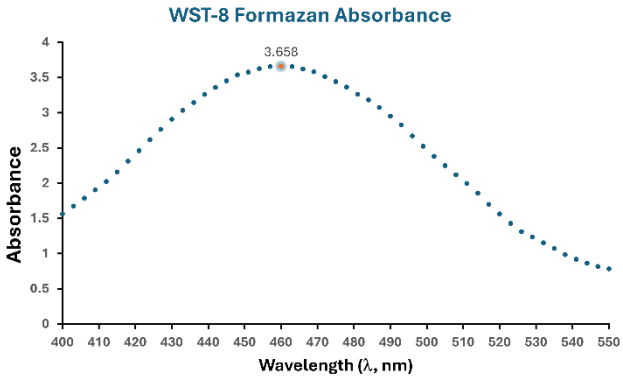

Figure 2.
Well plate max wavelength (λ) absorbance of WST-8 formazan analysis. The graph above illustrates the maximum absorbance peak of WST-8 solution (λmax = 460nm) after incubation (2h) with viable cells, indicating the conversion of WST-8 into soluble formazan dye.

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_2.

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

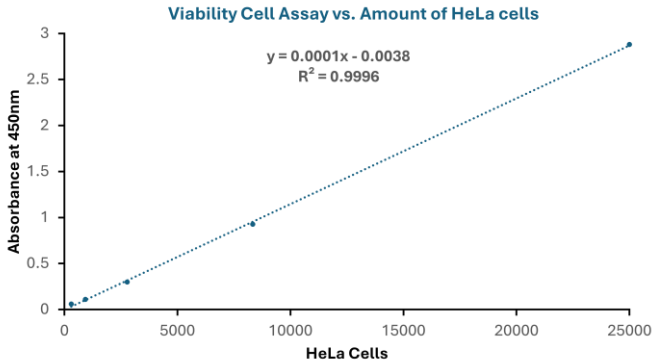

Figure 3.
WST-8 working solution cell viability vs. number of HeLa cells analysis.

References

1. Tominaga, H., Ishiyama, M., Ohseto, F., Sasamoto, K., Hamamoto, T., Suzuki, K., & Watanabe, M. (1999). A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Analytical Communications*, *36*(2), 47-50.
2. Kamiloglu, S., Sari, G., Ozdal, T., & Capanoglu, E. (2020). Guidelines for cell viability assays. *Food frontiers*, *1*(3), 332-349.
3. Ishiyama, M., Miyazono, Y., Sasamoto, K., Ohkura, Y., & Ueno, K. (1997). A highly water-soluble disulfonated tetrazolium salt as a chromogenic indicator for NADH as well as cell viability. *Talanta*, *44*(7), 1299-1305.
4. Chamchoy, K., Pakotiprapha, D., Pumirat, P., Leartsakulpanich, U., & Boonyuen, U. (2019). Application of WST-8 based colorimetric NAD (P) H detection for quantitative dehydrogenase assays. *BMC biochemistry*, *20*, 1-14.
5. Chen, C. H., Liao, Y. H., Muljadi, M., Lu, T. T., & Cheng, C. M. (2023). Potential Application of the WST-8-mPMS Assay for Rapid Viable Microorganism Detection. *Pathogens*, *12*(2), 343.
6. Lutter, A. H., Scholka, J., Richter, H., & Anderer, U. (2017). 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.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.