EasyAssay™ WST-1 Assay Kit

(3100 assays)

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CAT No. ZX-88103-3100

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Application:

Detection of Cell Viability, Proliferation & cytotoxicity

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Background

The Water-Soluble Tetrazolium salt-1 (WST-1) assay is an optimized colorimetric, non-radioactive assay for assessing cell viability, proliferation and cytotoxicity in mammalian cells based on their cellular redox potential. Dehydrogenase enzymes in metabolically active cells reduce the yellow highly water-soluble tetrazolium salt WST-1 in the presence of an electron carrier (1-methoxy-5-methylphenazinium methyl sulfate) to the brightly orange formazan dye which can be measured at 450 nm. Since this reduction can only occur in metabolically active cells, it is considered as an indicator of cell viability. The amount of produced formazan is proportional to the number of viable cells in the sample. The detection sensitivity of this assay is higher than the other tetrazolium salts such as MTT, XTT or MTS.

Intended use

Determining cell viability and proliferation.

Materials supplied in the Kit

Component	*Quantity*
WST-1	100 mg
HEPES	91.5 mg
1-Methoxy PMS solution (2.1 mg/mL)	1 mL
0.2 µM Filter	1

Storage instruction

WST-1 and 1-Methoxy PMS solution should be stored at -20°C.

Materials required but not supplied

Distilled water (dH_2O)

Precision pipettes and disposable filter pipette tips

Sterile clear 96 well plate flat bottom

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay preparation

i. **WST-1 Stock Solution:** Prepare WST-1 STCK SOLUTION by dissolving 100 mg WST-1 and 91.5 mg HEPES in 30 mL dH_2O, and Filter it via 0,2 µM filter. WST-1 stock solution can be stored at 4°C for up to 1 year, at -20°C for up to 2 years and at -80°C up to 3 years.

ii. **WST-1 Working solution:** add 1 mL of the 1-Methoxy PMS solution to 30 mL of WST-1 Stock Solution. WST-1 Working solution must be used immediately after preparation; it can also be stored at -20°C for up to 2 years. For small experiments make aliquots to avoid repeated freeze-thaws cycle.

Cell Proliferation and Cytotoxicity Assay

1. One to four days before the experiment, cultivate cells ($0.5x10^4$-$5x10^5$ cells/well) into a 96-well clear area plate containing 100 µL/well of cell culture medium. Each plate must be included at least three wells as Blank which contain only complete culture medium without the cells. Cultivate the cells in a CO_2 incubator at 37°C for 24-96 hours.

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of WST-1 assays.

2. Add 10 µL of the WST-1 Working solution to each well. Mix gently for one minute.
3. Incubate the cells for 30 minutes to 4 hours at 37°C in a CO_2 incubator.

- Optimize the incubation time for your experiment.

4. Shake the plate gently to evenly distribute the generated dye in the wells.
5. Read the absorbance signal at 450 nm (420-480 nm is acceptable).

Cell Counting assay

1. Prepare the cells in 96-well clear area plate containing 100 µL/well of cell culture medium. Each plate must be included at least three wells as Blank which contain only complete culture medium without the cells
2. Add 10 µL of the WST-1 Working solution to each well. Mix gently for one minute.

3. Incubate the cells for 30 minutes to 4 hours at 37°C in a CO_2 incubator.

➢ Optimize the incubation time for your experiment.

4. Shake the plate gently to evenly distribute the generated dye in the wells.
5. Read the absorbance signal at 450 nm (420-480 nm is acceptable).