TargetMol

A DRUG SCREENING EXPERT

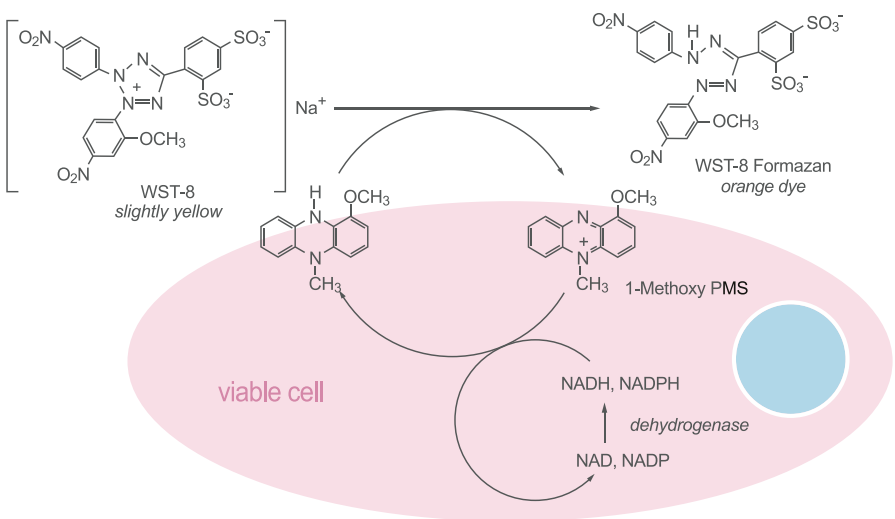
Cell Counting Kit-8 (CCK-8)

I Product Description

Cell Counting Kit-8 (CCK-8) allows convenient assays using WST-8 [2-(2-methoxy-4-nitrophenyl) -3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], which produces a water-soluble formazan dye upon reduction in the presence of an electron carrier, 1-Methoxy PMS. CCK-8 solution is added directly to the test cells with no pre-mixing of components required. CCK-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays.

II Assay Mechanism

WST-8 is reduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. The detection sensitivity is higher than any other tetrazolium salts such as MTT, XTT, MTS or WST-1.



Working mechanisms of Cell Counting Kit-8 (CCK-8)

III Advantages

- More sensitive than MTT, MTS, or WST-1
- Nontoxic to cells
- No organic solvents required
- Easy and stable one bottle solution; ready-to-use

IV Applications

- Cytotoxicity assays
- Cell viability assays
- Cytokine assays
- Cell proliferation determinations

V Storage and Stability

CCK-8 is stable for 2 years at 4°C with protection from light. Store at -20°C for longer-term storage.

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VI Experiment Procedure

1) Inoculate cell suspension (100µl/well) in a 96-well plate. Incubate the plate in an incubator (e.g., at 37°C, 5% CO2).

2) After removing the plate from the incubator, add 1/10 volume of CCK-8 solution directly to cells in culture medium. Mix thoroughly to achieve a homogenous solution by lightly tapping the outside of the plate several times while avoiding bubbles. For a 96-well plate, add 10 µl CCK-8 per 100 µl culture medium.

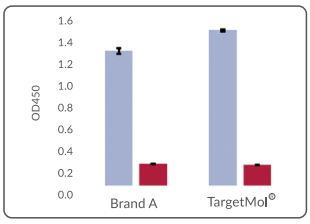
3) Incubate the plate in a cell culture incubator for 1 to 4 hours at 37°C until the color turns orange. Over incubation will yield false results.

4) Shake the plate on the lab shaker for 1 minutes to ensure the uniform coloration before reading.

5) Read the plate at a wavelength of 450nm and calculate the total cell activity based on the absorption value.

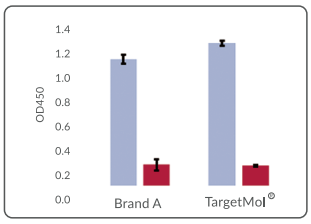
6) Optional: add 10 µl of 1 % SDS (dissolve 0.1 g SDS with PBS buffer to prepare 10 ml solution) directly to 100 µl of cells to stop the reaction. This allows the sample to be read up to 3 days without affecting the absorbance values.

VII Comparison of Product Effectiveness



【1】Cell line: HepG2

【2】Cell line: K562



【3】Cell line: H1299

Vehicle: 0.5% DMSO

Chemicals: 10 µM Drug X

Incubation:
37°C, 5% CO2, 2.5 hours

VIII Comparison of Detection Methods

Properties	MTT	XTT	WST-1	CCK8
Solubility of Formazan	-	+	+	+
Form	Powder	2 -bottle solution	solution	1-bottle solution
Preparation	Dissolve before use	Mix before use	Ready to use	Ready to use
Sensitivity	+	++	++	+++
Detection Speed	+	++	++	+++
Reading Wavelength	560~600nM	420~480nM	420~480nM	430~490nM
Cytotoxicity	+	-	-	-
Stability	+	-	+	+++
HTS Adaption	+	++	++	++
Convenience	+	++	++	+++

VI Note

1) CCK-8 is a ready-to-use solution. Mix the reagent to ensure a homogenous solution before use and avoid repeated freeze-thaws.

2) Pay attention to the edge effect of the 96-well plates. It is suggested to discard the outer surrounding plate wells and add a volume of PBS in its place.

3) This product is intended for R&D use only, not for any medical, household, or other uses.