

How to Normalize Reporter Gene Assays by the Use of the Cell Proliferation Reagent WST-1

Key words:

normalization of reporter gene assays, cell number measurement, measurement of cytotoxic effects

Gene expression in transfected eukaryotic cells is generally studied by linking a promotor sequence to an easily detectable “reporter” gene. Examples of such reporter genes are *firefly* luciferase, *E. coli* chloramphenicol acetyltransferase (CAT), or *E. coli* β-galactosidase (β-Gal).

In order to eliminate variances from the cell culture, results of individual wells have to be normalized with respect to protein concentration or cell number. Also, monitoring cell mass is often used as a measure of cytotoxic interferences.

Currently, in most research labs, normalization is performed by protein determination. These assays are time consuming, tedious, and inaccurate due to the many handling steps (standard curve, sample preparation, dilution, data processing, etc.) needed.

Easily measure cell number or cytotoxic effect

Cell Proliferation Reagent WST-1 is a ready-to-use reagent that quantitatively monitors the metabolic activity of cells. Conversion of WST-1 to a soluble formazan salt directly correlates with the cell number. The method requires neither washing nor harvesting of cells and the complete procedure can be performed in a tissue culture plate. In addition, an ELISA plate reader linked with an on-line computer allows rapid and automated data processing, which is advantageous especially for high throughput applications.

WST-1 assay procedure

1. Perform cell culturing and transfection according to your standard protocol.
2. 30-120 minutes before cell lysis, add 10% WST-1 reagent to the cell medium.
3. Quantify conversion of WST-1 directly, or from an aliquot using an ELISA reader.

4. Withdraw reagent/medium, and lyse cells for reporter gene assay.
5. Normalize reporter assay results according to the absorbance of the WST-1 assay.

Here are some data from experiments showing a comparison between normalization by protein determination and WST-1 reagent in combination with reporter gene assays.

These data show that WST-1:

- read-out is equivalent to the result of the protein assay,
- conversion is as sensitive as protein determination,
- read-out is equivalent to the result of the cell number,
- assay does not interfere with the outcome of the reporter gene assay.

Advantages of the WST-1 Reagent
sensitive and accurate
convenient
cost-saving
time-saving
all in one well

Experiment 1

WST-1 read-out is equivalent to the result of the protein assay (Figure 1). In a series of experiments, CHO-K1 cells were transfected with different plasmids, DNA amounts, and transfection reagents (A) in serum-containing medium or (B) under serum-free conditions. WST-1 reagent was applied for a period of 1 h just before cell lysis. After the incubation, 100 µl medium was transferred to a MTP, and absorbance was detected at 450/690 nm. Afterwards, 20 µl of the centrifuged cell lysates were used for protein determination according to the BCA method (MTP microassay).

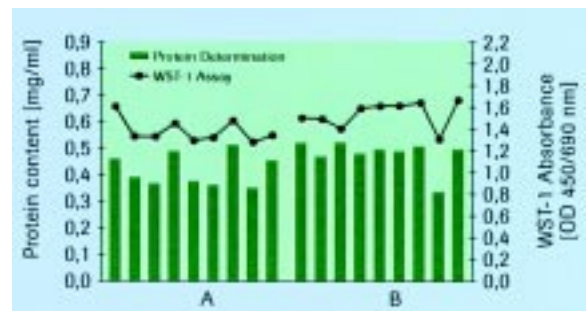


Figure 1: Comparison of WST-1 Assay and protein determination.

Result: WST-1 read-out is equivalent to the result of the protein assay.

Experiment 2

WST-1 conversion is at least as sensitive as protein determination (Figure 2). COS-7 cells were seeded in variable numbers in 24-well plates and cultured overnight. WST-1 was applied to the medium for 1 h and absorbance was measured at 450/690 nm. Medium was withdrawn, and the cells were lysed after washing with PBS. Protein determination was performed according to the BCA method.

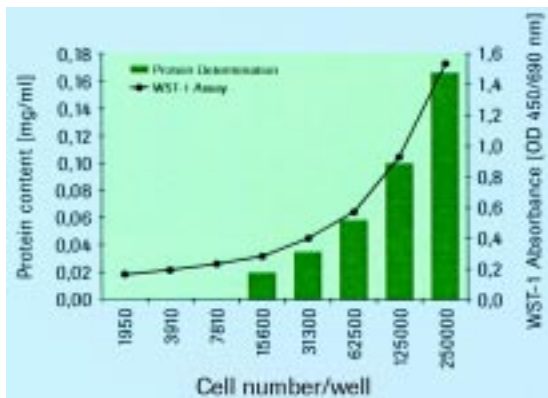


Figure 2: Comparison of WST-1 Assay and protein determination.

Result: WST-1 conversion is at least as sensitive as protein determination.

Experiment 3

WST-1 assay does not interfere with the outcome of the reporter gene assay (Figure 3). CHO-K1 cells were transfected with a β-Gal plasmid according to the standard protocol (6-well plate, 3/6 μl FuGENE™ 6 Transfection Reagent per 2 μg DNA). After 40 h, WST-1 was applied to the culture. Controls were not incubated with WST-1. After washing, cells were lysed and the reporter protein was quantified according to the β-Gal ELISA procedure.

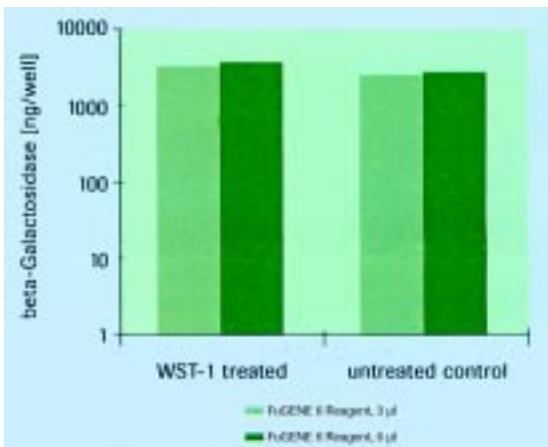


Figure 3: Combination of WST-1 Assay with the β-Gal ELISA.

Result: WST-1 assay does not interfere with the outcome of the reporter gene assay.

Experiment 4

WST-1 assay does not interfere with the outcome of the Luciferase Reporter Gene Assay, constant light signal (Figure 4). Since all cells are expressing luciferase, the WST-1 read-out correlates with the luciferase activity and therefore with the cell number. Stably transfected HepG2 cells, constitutively expressing firefly luciferase, were seeded in variable cell numbers into 96-well plates. After 24 h, cells were incubated with WST-1 for 1 h and absorbance was measured at 450/690 nm. Medium was withdrawn and luciferase activity was analyzed.

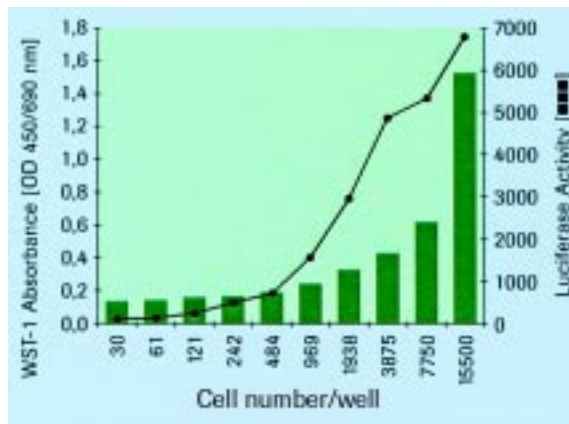


Figure 4: Combination of the WST-1 Assay with the Luciferase Reporter Gene Assay, constant light signal.

Result: WST-1 read-out correlates with the luciferase activity and therefore with the cell number, as all cells are expressing luciferase. The WST-1 assay does not interfere with the outcome of the Luciferase Reporter Gene Assay, constant light signal.



Product	Cat.No.	Pack Size
Cell Proliferation Reagent WST-1*	1 644 807	2500 tests
Luciferase Reporter Gene Assay, constant light signal	1 897 667	1000 tests
FuGENE™ 6 Transfection Reagent	1 814 443	1 ml
β-Gal ELISA	1 539 426	192 tests

* For research purposes only. Not for use in diagnostic procedures for clinical purposes. FOR IN VITRO USE ONLY.

This technical tip was originally published on our web site at:

<http://biochem.roche.com/techserv/ttip0598.htm>

