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A Geno Technology, Inc. (USA) brand name

CytoScan™ WST-1 Cell Cytotoxicity Assay

Colorimetric Assay for Quantitation of Cellular Cytotoxicity & Proliferation

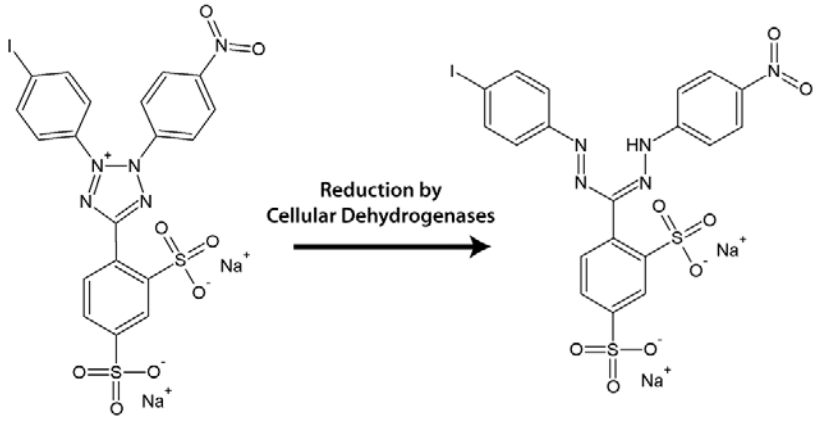
(Cat. # 786-212, 786-857)



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INTRODUCTION

CytoScan™ WST-1 Cell Cytotoxicity Assay is a sensitive and accurate assay for cell cytotoxicity and proliferation. The assay is highly convenient as it is performed in a single tissue culture well and requires no washing, harvesting or solubilization of cells. Adherent or suspension cells are cultured in a microplate and then incubated with WST-1 and the assay is monitored with a spectrophotometer. The assay principle is based upon the reduction of the tetrazolium salt WST-1 to formazan by cellular dehydrogenases (see figure below). The generation of the dark yellow colored formazan is measured at 420-480nm (optimal at 440nm) and is directly correlate to cell number. The kit components are sufficient for performing up to 500 or 2,000 assays.



WST-1
(pale yellow)

Formazan
(dark yellow)

ITEM(S) SUPPLIED

Description	Cat. # 786-212	Cat. # 786-857
WST-1 Tetrazolium Salt	1 vial	4 vials
CEC (CytoScan™ Electron Carrier)	1 vial	4 vials
WST Assay Buffer	6ml	30ml

STORAGE CONDITION

The kit is shipped at ambient temp. Store all kit components at 4°C, protected from light. The kit components are stable for six months, when stored as recommended.

PREPARATION BEFORE USE

1. Add 2.5ml Assay Buffer directly to each vial of WST-1 and CEC. Once dissolved the solutions should be stored at
   -20°C and protected from light.
2. Mix equal volumes of the WST-1 and CEC solutions to prepare the Assay Dye Solution before use. Once mixed the solutions should be stored at -20°C and protected from light.
   NOTE: *We recommend making aliquots before freezing, 1ml aliquots are sufficient for a 96 well plate. The WST-1/ CEC solution is stable for 3 months at -20°C.*

PROTOCOL

1a For a cytotoxicity assay, culture $5x10^4$-$5x10^5$ cells per well of a 96-well plate with a final volume of 100µl/well culture medium.

1b For a cell proliferation assay, culture $0.2x10^4$-$5x10^4$ cells per well of a 96-well plate with a final volume of 100µl/well culture medium. Prepare a blank well with culture medium only.
   NOTE: *The number of cells required depends on the individual cell type. The optimal linear range can be established by placing $5x10^3$-$5x10^5$ cells per well and following the protocol from step 3. Also the established linear standard curve can be used for cell counting by using a fixed incubation time in step 4.*
   NOTE: *Phenol red in the culture medium has little effect on the assay.*

2. Add the appropriate concentration/ volume of the test factors to be assayed and culture cells for 24-96 hours.
3. At the end of the treatments, add 10µl WST-1/ CEC Assay Dye Solution to each well. Gently shake the plate to mix chemicals with medium.
   NOTE: *If different volumes of culture media are used then adjust the volume of the WST-1/ CEC Assay Dye Solution accordingly.*
4. Incubate the plate for 30 min to 4 hours in the cell culture incubator.
   NOTE: *The cell culture time is dependent on cell type and concentration used, therefore determine the optimal times for your individual experiments.*
5. Shake the plate for 1 minute on an appropriate shaker and measure the absorbance using a microplate reader at 420-480nm and set the reference wavelength more than 600nm.
   NOTE: *The reaction can be stopped by the addition of 10µl 1% SDS.*
6. For cytotoxicity, subtract the culture medium background from your assay results and calculate percentage cytotoxicity with the following equation, using average absorbances for controls and experimental results.

   % Cytotoxicity= (100 x (Cell Control – Experimental)) ÷ (Cell Control)

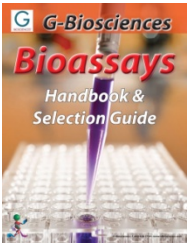
7. For cell proliferation the amount of absorbance is proportional to cell number. For cell counting a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

CITED REFERENCES

1. Li, H. et al (2009) Mol. Cancer Ther. 8: 3255 - 3265

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info.gbiosciences.com/complete-bioassay-handbook/

For other related products, visit our website at www.GBiosciences.com or contact us.

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