CytoScan™ SRB Cell Cytotoxicity Assay

(Cat. # 786-213)

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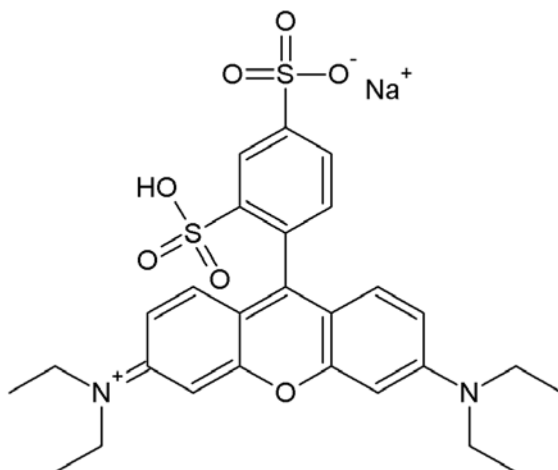
INTRODUCTION

CytoScan™ SRB Cell Cytotoxicity Assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by sulforhodamine B (SRB). This assay has been used for high-throughput drug screening at the National Cancer Institute (NCI)[1].

Sulforhodamine B is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides a sensitive linear response. The color development is rapid and stable and is readily measured at absorbances between 560 and 580nm. The kit components are sufficient for performing up to 1000 assays.



ITEMS(S) SUPPLIED (Cat. # 786-213)

Description	Size
SRB Dye (Sulforhodamine B)	0.4gm
Fixative Reagent	60ml
Dye Wash Solution 10X	100ml
SRB Solubilization Buffer	200ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Store all kit components at room temperature, protected from light. The kit components are stable for one year when stored as recommended.

ADDITIONAL ITEMS REQUIRED

- Microplate reader capable of readout between 550 and 580nm
- 96 well tissue culture plates

PREPARATION BEFORE USE

1. Dilute the Dye Wash Solution by adding 1 part 10X Dye Wash Solution to 9 parts distilled water. You require ~0.8ml per well to sufficiently wash the wells.
2. In a clean amber glass or plastic container, add 100ml 1X Dye Wash Solution. Remove 1ml 1X Dye Wash Solution and add to the SRB Dye vial, pipette up and down to resuspend and transfer the entire contents to the 100ml 1X Dye Wash Solution. Stir to mix. The SRB Dye Solution can be stored at room temperature protected from light. Crystals may form during storage, remove by filtering with a syringe filter prior to use in the assay.

PROTOCOL

The optimal conditions for monitoring cytotoxicity are to have the cells in the log phase of growth and not to exceed 10^6cells/cm^2. We recommend that each test is performed in a final volume of 200µl and includes a 200µl control sample of cell free medium to be used to blank absorbance readings.

> ***NOTE:** We recommend performing tests in serum-free media to reduce background where possible.*

1. Following treatment with appropriate test agents, remove the 96-well plate to a sterile tissue culture hood and gently layer 50µl Fixative Reagent onto each well.
   ***NOTE:** During and after the Fixative Reagent step, the plates should be disturbed as little as possible. Sudden shaking or jolting could dislodge cells and result in inaccuracies in protein quantity.*
2. Incubate the plate for 1 hour at 4°C.
3. Wash the wells 3-4 times with water and vigorously flick the plates between washes to remove excess water. The wash removes excess fixative and serum proteins.
4. Air dry the plates overnight if storage is required or incubate in a 45-50°C incubator for 30 minutes to remove excess wash.
5. Add 100µl SRB Dye Solution to cover the culture surface of the wells and incubate for 30 minutes at room temperature in the dark.
6. Repeat the wash step (Step 3) using 1X Dye Wash Solution instead of water.
7. Air-dry the plate as in Step 4.
   ***NOTE:** Once the SRB Dye Solution has been added the plates should be protected from light.*
8. Add 200µl SRB Solubilization Buffer to each well. Mix by pipetting up & down or gentle agitation to dissolve the dye completely.
9. Measure the absorbance at 550-580nm, 565nm is the absorption maximum, with a microplate reader.
   ***NOTE:** If intense color is visualized then utilize a suboptimal wavelength (490-530nm) to bring the readings within the linear instrumental range.*

CALCULATION OF RESULTS

Calculate the percent cytotoxicity for a given experimental treatment, by using the average absorbance values from experimental, cell control. Ensure that absorbances are blanked with the cell free medium controls.

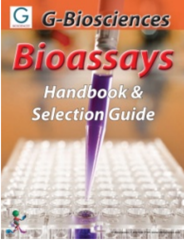
% Cytotoxicity= (100 x (Cell Control – Experimental)) ÷ (Cell Control)

REFERENCE

1. *Perez, R.P. et al (1993) Eur. J. Cancer. 29 A p395-399.*

RELATED PRODUCTS

Download our Bioassays Handbook.



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

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