G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

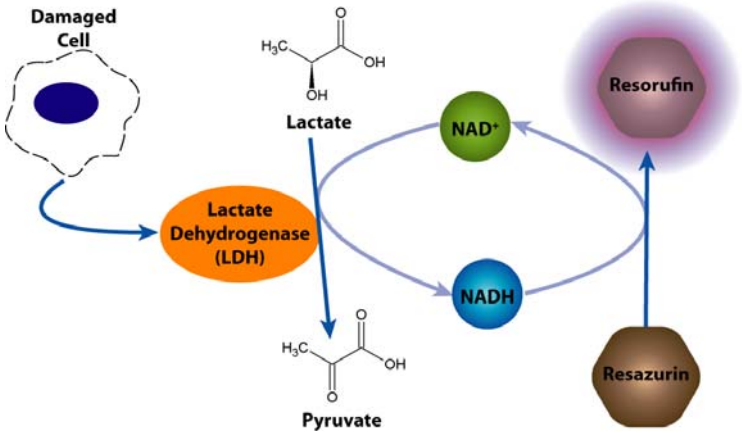
CytoScan™-Fluoro Assay

(Cat. # 786-211)

INTRODUCTION

Methods for determining lactate dehydrogenase (LDH) release, utilizing diaphorase have been used for measuring the cell cytotoxicity for many years. LDH activity is universally present in eukaryotic cells and the LDH enzyme activity is more stable than other enzymes present in a cell system. G-Biosciences's CytoScan™ -Fluoro Assay kit is a fluorometric method for estimating cell cytotoxicity and the number of non-viable cells. The Cytoscan™ -Fluoro kit measures the release of lactate dehydrogenase (LDH) from cells with damaged membranes. LDH released into the culture medium is measured with a coupled enzymatic assay that results in the conversion of a non-fluorescent compound (resazurin) to a fluorescent compound (resorufin), which can be detected by using a fluorometer.

The kit has been designed in such a way that it does not damage the healthy cells in vitro and the assay can be performed directly in the cell culture wells containing a mixed population of viable and damaged cells. The CytoScan™ -Fluoro Assay kit has its many usages in cell biology research and can be used for testing cell cytotoxicity or cell proliferation assays. The kit is supplied with ready-to-use buffers and solutions along with a detailed protocol.



Schematic representation of the principle of CytoScan™ -Fluoro Assay

ITEM(S) SUPPLIED (Cat. # 786-211)

Description	Size
CytoScan™ Substrate Mix	5 Vials
CytoScan™ -Fluoro- Lysis Buffer	1ml
CytoScan™ -Fluoro- Assay Buffer	60ml
CytoScan™ -Fluoro- Stop Solution	30ml
LDH Positive Control	30µl

STORAGE CONDITION

The kit is shipped at ambient temp. Store all kit components at 4°C, protected from light, except the CytoScan™ Substrate Mix and Assay Buffer store at -20°C. The kit components are stable for six months, when stored as recommended. The kit components are sufficient for performing >500 assays as per recommended protocols.

ADDITIONAL ITEM(S) REQUIRED

- Fluorescence plate reader with excitation 530-570nm and emission 580-620nm
- Multi-channel Pipettor
- 96 well tissue culture plates, compatible with fluorometer
- Culture plate shaker

PREPARATION BEFORE USE

All the kit components should be allowed to thaw and come to room temperature. Equilibrate the Substrate Mix and Assay Buffer to room temperature. Prepare Reaction Buffer by dissolving one vial of CytoScan™ Substrate Mix in 1ml of CytoScan™ -Fluoro Assay Buffer (protect from direct light) and add it to 10ml Assay Buffer in a light protected tube, total volume 11ml.

IMPORTANT INFORMATION

1. The CytoScan™ -Fluoro Assay Buffer may be further diluted in PBS before adding to the Substrate Mix as needed.
2. The resazurin dye present in the CytoScan™ -Fluoro Reaction Buffer and the resorufin formed during the assay are light sensitive. Prolonged exposure of the reagents to light will result in increased background fluorescence in the assay and decreased sensitivity.

ASSAY CONTROLS

The following assay controls are recommended for each assay plate set up:

No Cell Control	Untreated Cells Control	Maximum LDH Release Control
No Cell Control may be set up in Triplicate wells without the cells. This will serve as the negative control to determine background fluorescence that might be present.	Untreated Cells Control may be set up in triplicate wells with untreated cells. This will serve as a vehicle control. Same solvent used to deliver the test compound may be added to the vehicle control wells.	Maximum LDH Release Control may be set up in triplicate wells. Add 2µl CytoScan™ -Fluoro Lysis Buffer to the positive control wells before addition of CytoScan™ -Fluoro Reaction Buffer.

GENERAL RECOMMENDATIONS FOR THE ASSAY:

Correction of Background fluorescence generated by serum LDH

Tissue culture medium may contain significant amount of LDH from animal serum, which can generate background fluorescence. This can be corrected by developing a control to measure the fluorescence from supplemented culture medium in the absence of cells. Reduced serum concentrations or serum free culture medium can also be used to reduce or eliminate background fluorescence resulting from LDH present in the serum.

100% Cell Lysis Control

Researchers performing Cytotoxicity assay may also require a 100% cell lysis control to determine the maximum LDH present. Use of recommended dilution of Lysis Buffer provided in the kit will result in almost immediate lysis of most cell types and cytoplasmic LDH release into the surrounding culture medium.

LDH Positive Control

LDH positive control is also supplied with the kit, if a researcher prefers to perform a positive control assay. LDH dilution 1:5000 to 1:10,000 in PBS containing 1% BSA or LDH dilution decided by the researcher may be used.

PROTOCOL

A. Cytotoxicity Assay Protocol:

1. First set up the wells containing cells in culture medium in a 96 well plate.
2. In the next step, add test compounds and vehicle controls to appropriate wells to a final volume of 100µl in each well. Culture cells for desired test compound exposure period.
3. Remove the assay plates from 37°C incubator and equilibrate to 22°C for about 20-30 minutes.
4. Optional Step: Add 2µl of CytoScan™ -Fluoro- Lysis Buffer per 100µl original volume to the positive control wells, if the Lysis Buffer is used to generate a maximum LDH Release Control. However, if a larger pipetting volume is required, use 10µl of a 1:5 diluted CytoScan™ -Fluoro- Lysis Buffer.
5. Add 100µl of Reaction Buffer to the wells containing 100µl medium with cells and mix it. Incubate the culture plate at 22°C for 10 minutes.
6. Add 50µl of CytoScan™ -Fluoro- Stop Solution to each well. This step is recommended for consistency, however it is optional.
7. Shake the plate for 10-15 seconds and record the fluorescence with an excitation wavelength of 560nm and an emission wavelength of 590nm.

CALCULATION OF RESULTS

1. Subtract the average fluorescence reading of the culture medium background from all fluorescence values of experimental wells.
2. Calculate the percent cytotoxicity for a given experimental treatment, by using the average fluorescence values from experimental, maximum LDH release, and culture medium background.

$$\% \text{ Cytotoxicity} = \frac{100 \times (\text{Experimental} - \text{Culture Medium Background})}{\text{Maximum LDH Release} - \text{Culture Medium Background}}$$

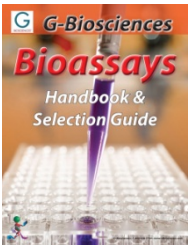
B. Cell Proliferation Assay Protocol

The CytoScan™ -Fluoro Assay can also be used for estimation of the total number of cells in assay wells at the end of a proliferation assay. The method involves lysing all the cells to release LDH enzyme followed by adding the CytoScan™ -Fluoro Reaction Buffer.

1. To appropriate wells of a 96 well plate, add the test compound and vehicle controls so that the final volume is 100µl in each well. Culture cells for desired test compound exposure period.
2. For each 100µl original volume, add 2µl of CytoScan™ -Fluoro- Lysis Buffer. [Note: If a larger pipetting volume is required, use 10µl of a 1:5 diluted CytoScan™ -Fluoro-Lysis Buffer].
3. Remove the assay plates from 37°C incubator and equilibrate to 22°C for about 20-30 minutes.
4. After equilibration at 22°C, add 100µl of CytoScan™ -Fluoro Reaction Buffer to 100µl medium containing cells and incubate at 22°C for 10 minutes.
5. Shake the plate for 10 seconds and record fluorescence with an excitation wavelength of 560nm and an emission wavelength of 590nm.
6. The total number of cells present will be directly proportional to the background-subtracted fluorescence values, which represent LDH enzyme activity.

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.

Last saved: 10/4/2012 CMH

www.GBiosciences.com