Promega

Product Application

LDH-Glo™ Cytotoxicity Assay with 3D (Spheroid) Microtissues

Measure induced LDH released into cell culture media from 3D microtissues using the LDH-Glo™ Cytotoxicity Assay. Remaining microtissues can then be assayed for viability with the CellTiter-Glo® 3D Cell Viability Assay.

Kit: LDH-Glo™ Cytotoxicity Assay (Cat.# J2380)

Analysis: Luminescence

Sample Type: 3D human colorectal carcinoma (HCT116) microtissues

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information see Technical Manual TM548, available at: **www.promega.com/protocols**

or contact Technical Services at **techserv@promega.com**

Materials Required:

- LDH-Glo™ Cytotoxicity Assay (Cat.# J2380)
- Opaque white multiwell plate (e.g., Corning Costar® #3917 96-well)
- Luminometer (e.g., GloMax® Discover System (Cat.# GM3000)
- LDH Storage Buffer (200mM Tris-HCl (pH 7.3), 10% Glycerol, 1% BSA)
- Optional: CellTiter-Glo® 3D Cell Viability Assay (Cat.# G9681)
- Optional: Orbital plate shaker

Protocol:

1. Add test compound or vehicle control to prepared microtissues. Incubate for desired time at desired conditions.
2. Remove small amounts of media (2-5µl) from each treated spheroid well at desired timepoints. Take care to not touch or disturb the spheroids.
3. Dilute media in LDH Storage Buffer (1:20). If assay will be delayed, freeze the diluted media samples at -20°C.
4. On the day of assay, thaw the collected samples. Further dilute in LDH Storage Buffer to be within linear range of the assay (e.g., 1:100).
5. Prepare the LDH Detection Reagent as specified in the Technical Manual (TM548). Ensure the Reductase Substrate is placed on ice before LDH Detection Reagent preparation.
6. Add 50µl of prepared reagent to 50µl of sample in an opaque white 96-well plate.
7. Incubate for 60 minutes at room temperature.
8. Read luminescence with a 0.3 second integration time using a luminometer.

Optional:

9. Prepare CellTiter-Glo® 3D Reagent as specified in the Technical Manual (TM412). Ensure reagent and cells are equilibrated to room temperature.
10. Add a volume of CellTiter-Glo® 3D reagent equal to the volume of the cell culture medium remaining in each well (subtract the total volume of media removed for LDH measurement from the starting volume of media).
11. Incubate with shaking (700rpm) with a lid at room temperature for 30 minutes (may need to adjust shaking/incubation time based on cell culture system used).
12. Read luminescence with a 0.3 second integration time using a luminometer.

Results:

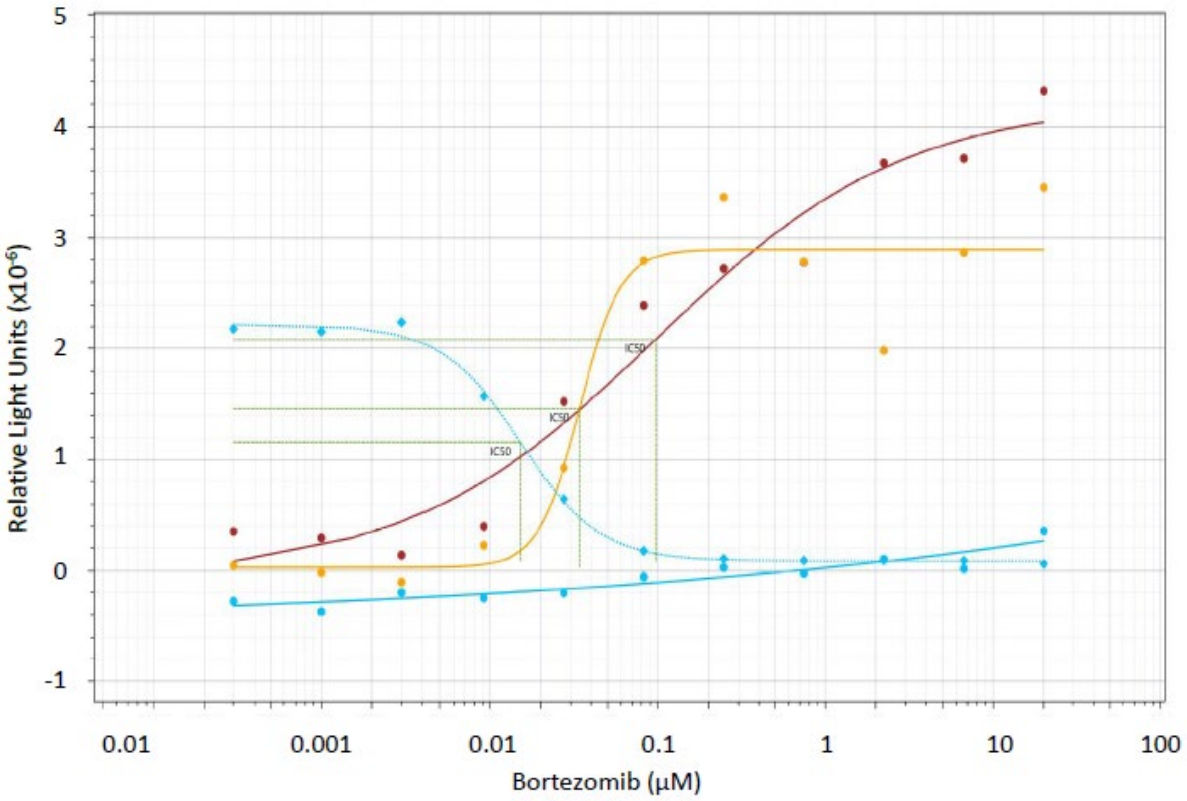

Figure 1. Dose-Response Curves Generated using LDH-Glo™ Cytotoxicity Assay (Cat.# J2380) from 3D (Spheroid) HCT116 microtissues multiplexed with CellTiter-Glo® 3D Viability Assay (Cat.# G9681) signals. HCT116 spheroids were grown in ULA plates for 3 days at 37°C, 5% CO_2 and subsequently treated with Bortezomib (37°C, 5% CO_2) to induce LDH release. Media samples were taken from the spheroid plate at 24 hours, 48 hours and 64 hours, diluted with LDH Storage Buffer. The diluted samples were assayed with LDH-Glo™ Cytotoxicity Assay. Spheroids were then assayed with CellTiter-Glo® 3D Viability Assay. Both plates were read on a GloMax® Discover System (Cat.# GM3000). The dose response curves were generated using the GloMax® Analysis Software. The image above displays the resulting dose response curves. The LDH curves are represented with circles: blue at 24 hours, red at 48 hours and yellow at 64 hours. The CellTiter-Glo curve is represented with blue diamonds and a dotted line as a single endpoint curve.