CytoTox 96® Non-Radioactive Cytotoxicity Assay

INSTRUCTIONS FOR USE OF PRODUCT G1780.

Quick PROTOCOL

Cell-Mediated Cytotoxicity Assay

Assay Plate Setup with Controls

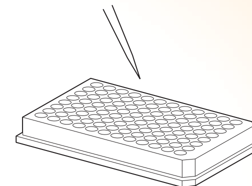
1. Using a round- or V-bottom 96-well culture plate, set up the following control and experimental wells:
   a. Effector cells (at each effector cell concentration) for Effector Cell Spontaneous LDH Release Control.
   b. Constant number of target cells and varying number of effector cells to experimental wells.
   c. Cells for Target Cell Spontaneous LDH Release Control.
   d. Cells for Target Cell Maximum LDH Release Control.
   e. Culture medium and Lysis Solution (10X) for Volume Correction Control.
   f. Culture medium for Culture Medium Background Control.
2. Centrifuge the plate at 250 × *g* for 4 minutes.

Cell Culture and Supernatant Harvest

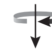
1. Incubate the plate at 37°C for 4 hours (or an empirically determined time period).
2. Forty-five minutes prior to supernatant harvest, add Lysis Solution (10X) to Target Cell Maximum LDH Release Control.
3. Centrifuge the plate at 250 × *g* for 4 minutes.

LDH Measurement

1. Transfer 50µl of the supernatant from each well of the assay plate to the corresponding well of a flat-bottom 96-well enzymatic assay plate.
2. **(Optional)** Add 50µl of a 1:5,000 dilution of LDH Positive Control to separate well(s).
3. Reconstitute Substrate Mix using Assay Buffer. Add 50µl of the reconstituted Substrate Mix to each well of the plate.
4. Cover the plate and incubate at room temperature, protected from light, for 30 minutes.
5. Add 50µl of the Stop Solution to each well of the plate.
6. Record absorbance at 490nm.

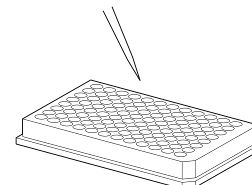


Prepare assay plate with controls (use round- or V-bottom 96 well plate).

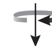


Centrifuge.

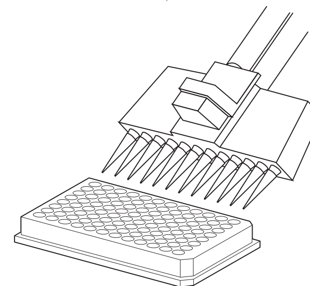
Incubate at 37°C.



Add Lysis Solution (10X) to Target Cell Maximum LDH Release Control.

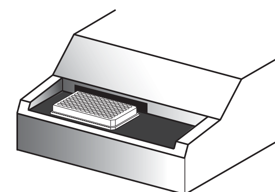


Centrifuge.



Transfer to flat-bottom 96 well plate. Add Substrate Mix.

Cover and incubate. Add Stop Solution.



Record absorbance at 490nm.

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Calculation of Results

1. Compute corrected absorbance values.
2. Use the corrected values in the following formula to compute percent cytotoxicity for each effector:target cell ratio.

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental} - \text{Effector Spontaneous} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Target Spontaneous}} \times 100$$

See additional protocol information in Technical Bulletin #TB163, available online at: **www.promega.com/tbs**

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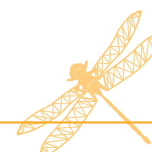

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CytoTox 96® Non-Radioactive Cytotoxicity Assay

INSTRUCTIONS FOR USE OF PRODUCT G1780.

Quick PROTOCOL

Total Cell Number Assay

Assay Plate Setup

1. To a 96-well culture plate, add cells to the experimental wells and culture medium to separate wells for background control.

Cell Culture and Supernatant Harvest

1. Treat cells with compound(s) to be tested, and incubate for the desired test period (include a no-treatment control).
2. Add Lysis Solution (10X) to **all** wells.
3. Incubate for 45–60 minutes at 37°C.

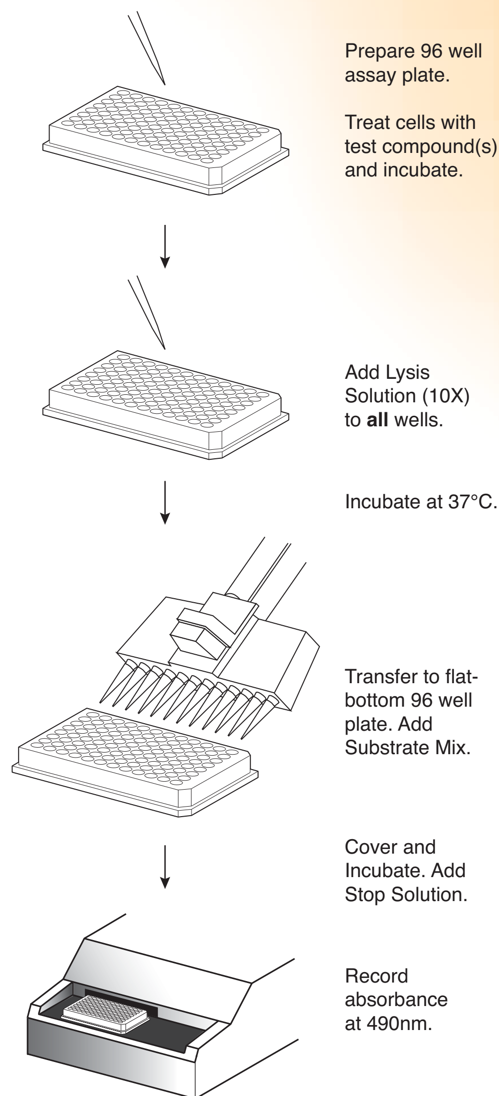
LDH Measurement

1. Transfer 50µl of the supernatant to each well of a flat-bottom 96-well enzymatic assay plate.
2. **(Optional)** Add 50µl of a 1:5,000 dilution of LDH Positive Control to separate well(s) containing no cells.
3. Reconstitute Substrate Mix using Assay Buffer. Add 50µl of the reconstituted Substrate Mix to each well of the plate.
4. Cover the plate and incubate at room temperature, protected from light, for 30 minutes.
5. Add 50µl of the Stop Solution to each well of the plate.
6. Record absorbance at 490nm.

Calculation of Results

The number of cells present will be directly proportional to the absorbance values, which represent LDH activity. Resulting data can be plotted with cell number along the X axis and absorbance 490nm values along the Y axis.

See additional protocol information in Technical Bulletin #TB163, available online at: **www.promega.com/tbs**



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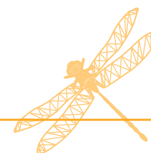

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