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LDH cytotoxicity assay

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We use this protocol and it's working

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Abstract

This protocol is intended for use with the CytoTox 96® Non-Radioactive Cytotoxicity Assay kit from ProMega (Cat #G1780).

The CytoTox 96® Non-Radioactive Cytotoxicity Assay is a colorimetric alternative to 51Cr release cytotoxicity assays. The CytoTox 96® Assay quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis, in much the same way as 51Cr is released in radioactive assays. The half-life of LDH that has been released from cells into the surrounding medium is approximately 9 hours. Released LDH in culture supernatants is measured with a 30-minute coupled enzymatic assay, which results in the conversion of a tetrazolium salt (iodonitrotetrazolium violet; INT) into a red formazan product. The amount of color formed is proportional to the number of lysed cells. Visible wavelength absorbance data are collected using a standard 96-well plate reader. Methods for measuring LDH using tetrazolium salts in conjunction with diaphorase or alternate electron acceptors have been used for many years (1). Variations on this technology have been reported for measuring natural cytotoxicity and are identical (within experimental error) to values determined in parallel 51Cr release assays (2,3).

Attachments

PDF

cytotox-96-nonradioa...

984KB

Materials

Materials to Be Supplied By the User
• 96-well culture plates compatible with a standard plate reader
• multichannel pipettor
• reservoirs to hold CytoTox 96® Reagent and Stop Solution
• plate reader capable of recording absorbance 490 or 492nm
• plate shaker

G1780 contains sufficient Substrate Mix, Assay Buffer and Stop Solution for 1,000 cell-mediated cytotoxicity assays in 96-well plate format. Includes:
• 5 vials Substrate Mix
• 60ml Assay Buffer
• 25µl LDH Positive Control
• 5ml Lysis Solution (10X)
• 65ml Stop Solution

Storage Conditions: Store Substrate Mix and Assay Buffer frozen at –20°C protected from light. CytoTox 96® Reagent (Assay Buffer combined with Substrate Mix) may be stored for 6–8 weeks at –20°C protected from light without loss of activity. Store LDH Positive Control, Lysis Solution (10X) and Stop Solution at 4°C. CAT.# G1821
Note: Upon storage, a precipitate might form in the Assay Buffer. This precipitate does not affect assay performance. The precipitate may be removed by centrifugation at 300 × g for 5 minutes. Use 12ml of the supernatant to reconstitute the Substrate Mix.

1 Thaw Assay Buffer, remove 12ml and promptly store the unused portion at –20°C. Warm 12ml of Assay Buffer to room temperature; keep protected from light. Add 12ml of room-temperature Assay Buffer to a bottle of Substrate Mix to form the CytoTox 96® Reagent. Invert and shake gently to dissolve the substrate.

1.1 Notes:
1. A 37°C water bath may be used to thaw the Assay Buffer, but return the Assay Buffer to –20°C storage protected from light as soon as it is thawed and used.

2. Upon storage, a precipitate might form in the Assay Buffer. This precipitate does not affect assay performance. The precipitate may be removed by centrifugation at 300 × g for 5 minutes. Use 12ml of the supernatant to reconstitute the Substrate Mix.

3. One bottle of CytoTox 96® Reagent is enough for two 96-well plates (200 assays) when using 50µl samples.

4. Once resuspended, protect the CytoTox 96® Reagent from strong direct light and use immediately. Store any unused Reagent at –20°C.

2 Recommended Controls:

Perform each of these controls on each plate being assayed.

No-Cell Control: Set up triplicate wells without cells to serve as the negative control to determine culture medium background.

Vehicle-Only Cells Control: Set up triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds to the vehicle control wells.

***Maximum LDH Release Control (Optional but Recommended!): Set up triplicate wells to determine the Maximum LDH Release Control. Add 10µl of 10X Lysis Solution per 100µl of Vehicle-Only Cells Control 45 minutes before adding CytoTox 96® Reagent. Note: This control is only required if calculating % cytotoxicity

3 Set up 96-well assay plates containing cells in culture medium. Be sure to prepare wells for the recommended controls.

4 Add test compounds and vehicle controls to appropriate wells so the final volume is 100–150µl in each well.

5 Incubate cells at 37°C for the desired test exposure period.

6 If Lysis Solution is used to generate a Maximum LDH Release Control, add 10µl of 10X Lysis Solution (per 100µl original volume) to the positive control wells 45 minutes before adding CytoTox 96® Reagent.

7 Transfer 50µl aliquots from all test and control wells to a fresh 96-well flat clear bottom plate.

8 Add 50µl of the CytoTox 96® Reagent to each sample aliquot. For 384-well format, add 12.5µl to each sample aliquot. Cover the plate with foil or an opaque box to protect it from light and incubate for 30 minutes at room temperature.

9 Add 50µl of Stop Solution to each well of the 96-well plate.

10 Pop any large bubbles using a syringe needle, and record the absorbance at 490nm or 492nm within 1 hour after adding the Stop Solution.

11 To calculate results:

Subtract the average values of the culture medium background from all values of experimental wells.

Use the corrected values in the following formula to compute percent cytotoxicity:
Percent cytotoxicity = 100 × Experimental LDH Release (OD490) Maximum LDH Release (OD490)