APExBIO
An Apoptosis and Epigenetics Company

Product Information

LDH-Cytotoxicity Colorimetric Assay Kit

I. Kit Contents:

Components	K2025-400 400 assays	Part Number
Catalyst (lyophilized)	1 vial	K2025-C-1
Dye Solution	45 ml	K2025-C-2

II. Introduction:

Cells treatment with the cytotoxic compound may undergo necrosis, in which they lose membrane integrity. Hence cytotoxicity can be detected by the quantification of plasma membrane damage. Lactate dehydrogenase (LDH) is a stable enzyme exists in nearly all living cells. When damage of the plasma membrane, LDH is rapidly released into the cell culture supernatant.

The LDH-Cytotoxicity Colorimetric Assay Kit provides a simple and fast way for the quantification of cytotoxicity based on the measurement of activity of LDH released from damaged cells, which can be detected by a coupled enzymatic reaction: LDH oxidizes lactate to pyruvate which reacts with tetrazolium salt INT to form formazan. The formazan dye is water-soluble and can be detected by spectrophotometer at 500 nm. The assay is convenient, precise and sensitive, and is suitable for a variety of cytotoxicity studies and only takes ~0.5-1 h.

III. Preparation of Working Solutions:

Reconstitute the Catalyst in 1 ml ddH_2O for 10 min and mix thoroughly. The Catalyst Solution is stable for several weeks at 4℃.

After thaw, the Dye Solution is stable for several weeks at 4℃. Avoid freeze/thaw cycles.

Preparation of Reaction Mixture: For 100 assays, mix 250 µl of Catalyst Solution with 11.25 ml of Dye Solution. The mixture solution should be prepared immediately before use.

IV. LDH-Cytotoxicity Assay Protocol:

1. Collect cells (adherent or suspension) and wash 1X with assay medium (e.g., medium containing 1 % serum or 1 % BSA).
   Note: Trypsin may be used to remove adherent cells from a culture surface.
2. Preparing the following samples individually in a 96-well plate:
   Background Control: Add 200 µl medium/well into triplicate wells. The background value has to be subtracted from all other values.
   Low Control: Add 1 - 2 x 10^4 cells/well in 200 µl assay medium into triplicate wells.
   High Control: Add 1 - 2 x 10^4 cells/well in 200 µl assay medium containing 1% Triton X-100 into triplicate wells.
   Test Sample: Add 1 - 2 x 10^4 cells/well in 200 µl assay medium containing test substance into triplicate wells.
3. Incubate cells in an incubator (5 % CO_2, 90 % humidity, 37℃) for the appropriate time of treatment determined for test substance.
4. Centrifuge the cells at 250 g for 10 min.
5. Transfer 100 µl/well supernatant carefully into corresponding wells of an optically clear 96-well plate.
6. Add 100 µl Reaction Mixture to each well and incubate for up to 30 min at room temperature. Protect the plate from light.
7. Measure the absorbance of all samples at 490 - 500 nm using a microtiter plate reader. The reference wavelength should be more than 600 nm.

V. Calculation of the Percentage Cytotoxicity:

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Cytotoxicity (%) = (Test Sample - Low Control) / (High Control - Low Control)X 100

Frequently Asked Questions

1. Is it OK to use 540 nm filter for reading?

540 nm can read ~50% signal, so as long as you have good readings, it's fine.

2. Can medium be stored at -20℃ before assay, for how long?

Yes, medium can be stored frozen for at least 2 months.

3. Can reagents be stored at -20℃ after reconstitution?

The reconstituted Catalyst solution is not stable under frozen conditions.

4. What kinds of readings are expected for low and high controls?

Low control: OD 0.5 - 1.0 and High control: OD 2 - 3.

5. What is the Sensitivity Limit?

0.05 U/ml.

6. Does phenol red affects the assay?

Phenol red will increase the OD reading (0.1 - 0.2). However, if your background, control media and testing media all contain the same amount of phenol red, the affect can be ignored, since it will be subtracted from your testing samples.

7. Does serum affects the assay?

Serum will increase the OD reading (0.5). However, if your background, control media and testing media all contain the same amount of serum, the affect might be ignored (depending on each situation), since it will be subtracted from your testing samples.

8. I got same value in high and control samples. Why?

The high control is treated with 1% Triton X100, thus cells will release LDH into media, whereas low control only control regular medium (not much LDH will be released). The differences should be significant. There are several possibilities that I can think of:

1) Maybe the cells do not contain LDH;

2) Maybe the Triton X-100 concentration was not correct, or the low control cells were also died. Generally, low control should have an OD reading 0.5-1, whereas high control has an OD reading 2-3.

3) The machine filter setting might not be correct.

9. Why do I have a high background? What can I do to lower it?

The high background could be attributed to two factors:

The culture medium contains high LDH, probably from the serum.

The high LDH could be from leaky cells.

The remedy could be: Reduce the serum concentration to 0.5%. Since there is always some cells that will leak LDH, reduce the number of cells in the assay. By decreasing the serum concentration or the number of cells or even both you will lower the control reading.

10. Does pyruvate present in the DMEM medium interfere with LDH reaction?

We used excessive amount of lactate (~ 100mM) in the reaction system, which will promote LDH catalyzed conversion of lactate to pyruvate, and inhibit the conversion of pyruvate to lactate. So the pyruvate in the medium should not affect the assay.

11. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

12. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

13. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

14. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

15. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

16. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

17. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

18. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

19. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, It is recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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