Product Information

LDH-Cytotoxicity Colorimetric Assay Kit II

I. Kit Contents:

Components	K2027-500 500 assays	Cap Color	Part Number
WST Substrate Mix	1 vial	Amber	K2027-C-1
LDH Assay Buffer	50 ml	NM	K2027-C-2
Cell Lysis Solution	5 ml	Clear	K2027-C-3
Stop Solution	5 ml	Clear	K2027-C-4
LDH	Lyophilized	Red	K2027-C-5

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 II 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 produce NADH, which then reacts with WST to generate yellow color, which can be easily detected by plate reader or spectrophotometer at OD450 nm. The intensity of the yellow color correlates directly with the cell number lysed. Since WST is more stable, the reaction can be read multiple times and stopped at any time point during the reaction. Since WST is brighter, the assay needs less amount of culture medium and thus significantly reduced the background from culture medium and serum. In this assay, cells can be cultured in regular 10% serum containing medium without special medium or reducing serum is required. The kit also provides LDH positive control. Assay takes less than 1 hour.

III. Preparation of Working Solutions:

Reconstitute the WST Substrate Mix in 1.1 ml ddH2O for 10 min and mix thoroughly. The solution is stable for two month at 4℃.

Preparation of LDH Reaction Mix: For 100 assays, mix 200 µl of WST Substrate Mix with 10.0 ml of LDH Assay Buffer. The LDH Reaction Mix should be stable for several weeks at 4℃.

IV. LDH-Cytotoxicity Assay Protocol:

1. Collect cells (adherent or suspension) and wash once with fresh regular culture medium, then seed 100 µl cells (with 2-10 x 10^4 cells) in a 96-well plate as the following:

Background Control: 100 µl culture medium per well in triplicates with no cells. The Background Control will measure reagents and LDH background from culture medium serum. The background value has to be subtracted from all other values.

Low Control: 100 µl cells in triplicate wells.

High Control: 100 µl cells in triplicates, add 10 µl Cell Lysis Solution each well, mix. To adjust the increase of medium volume, 11 µl of the medium may be used in LDH activity assay at step 4.

Test Sample: 100 µl cells in triplicates, add test substances each well, mix.

Notes:

a) Trypsin may be used to remove adherent cells from a culture surface before seeding in a 96-well plate.

b) The amount of cells to be used per well depends on the cell types. To optimize the assay, you can do a quick testing by using 2, 4, 8 x 10^4 cells per well, and then follow the assay protocol to determine the cell number you should use. The high control should be OD_{450nm} ~ 2.0 after 30 min treatment with 10 % Cell Lysis Solution, while the low control should be OD450nm < 0.8. The reaction time should be set at ~ 30 min.

c) Positive control (1 µl LDH) can be used to test whether all reagents are working properly to response to active LDH enzyme.

d) If the test substances are not dissolved in PBS, a solvent control may be performed by addition of the same amount of solvent in triplicates without testing substances.

2. Incubate cells in an incubator (5 % CO_2, 90 % humidity, 37℃) for the appropriate time of treatment determined for test substance. Gently shake the plate at end of the incubation to ensure LDH is evenly distributed in the culture medium.

3. Centrifuge cells at 600 x g for 10 min to precipitate the cells.

4. Transfer the clear medium solution (10 µl/well) into an optically clear 96-well plate.

5. Add 100 µl LDH Reaction Mix to each well, mix and incubate for 30 min at room temperature.

6. Measure the absorbance of all controls and samples with a plate reader equipped with 450 nm (440 - 490 nm) filter. The reference wavelength should be 650 nm.

Notes:

a) The reaction time can be decreased or increased depend on the color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be OD450nm ~ 2.0, while the low control should be OD450nm < 0.8.

b) The reaction can be stopped by adding 10 µl of Stop Solution, mix and read within 48 hours without significant changes. Protect the reaction from light and evaporation.

V. Calculation of the Percentage Cytotoxicity:

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

LDH Cytotoxicity Assay Kit II. Jurkat T cells were cultured in 96-well plate in 100 µl of culture medium. LDH Assay was performed using 10µl of culture medium using the WST probe. Low control (white bar); High control (black bar).

Frequently Asked Questions

1. We would like to know if this kit can be used for supernatant samples that have been frozen?

It is best to use this with fresh samples rather than frozen. The protocol will have to be changed slightly to accommodate the frozen samples. Also, the success of the assay would depend on numerous extra criteria including how well the samples were frozen, how long they were frozen, number of times they were thawed out etc, which increases the complexity. Therefore it is recommend to stick with fresh samples.

2. My cells are primarily cultured chick embryonic forebrain neurons co-grown with glial cells. I just need to know whether my culture is healthy in general along their life time in culture. Would you please advise whether your kit is appropriate for my purpose? If, yes, how? the plating density of the cells is fixed at 3000 cells/mm^2. Do I have to plate the cells using a 96-well plate? Can I use 24-well, 12-well plate, and a microelectrode array chamber, then at day 7,14,21, and 28, she could take out some supernatant to a 96-well plate to assay? if this is ok, may I store the supernatants taken out on different days, store them at -20°C or -80°C, then perform the LDH assay at one time?

Yes, this kit would be appropriate for this assay and we have explained below how.

For this particular kit, you can actually look at the LDH released into the cell media in her 7 - 28 day cultures which we assume would be very low relative to the high controls, which would indicate that the cells are viable. Also, by doing a comparative LDH level analysis between the different time points the client would be able to see by which day/time the cells release lowest LDH and have highest viability. We would not store samples. Just do a fresh testing at the different day time points. You grow cells in any size plate, but would need to have them in a 96 well plate for the expt. If you want to take just the sup, you can, but then all the controls also have to be taken appropriately. For eg, for the high cont., taking the sup and

treating with cell lysis solution is not going to do the trick. You need the cells for that. The number of cells for each assay will also need to be optimized as mentioned in the protocol.

3. We have a customer who is having a little difficulty using the LDH-Cytotoxicity Assay Kit II. The positive control is yielding an OD of 1.5 but the low and high control are both showing an OD of 0.6. Please could you advise as to the likely cause of this result.

To begin with, the client is using very few cells. We recommend the cell number to be within the range of 2 - 8 x10 ^{4}. Please request the client to increase their cell number. The amount of cells to be used per well depends on the cell types. To optimize the assay, you can do a quick testing by using 2, 4, 8 x 10^4 cells per well, and then follow the assay protocol to determine the cell number you should use. The high control should be OD_{450nm} ~ 2.0 after 30 min treatment with 10 % Cell Lysis Solution, while the low control should be $OD4_{50nm}$ < 0.8. The reaction time should be set at ~ 30 min. Once this is done, their issue with the ODs of HC vs LC will be resolved.

They will need to optimize the dose and incubation time with the drug, to ensure that they get efficient results. Additionally they might have to do a solvent control also if they do not use PBS with their drugs.

At what OD did they read the plate? Also, The reaction time can be decreased or increased depend on the color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be OD_{450nm} ~ 2.0, while the low control should be OD_{450nm} < 0.8.

4. Might this assay be negatively effected by pan hematin or $SnCl_2$? These are present in my samples.

Since this is a relative assay with the inclusion of the high and low controls, which will have the same pan hematin or $SnCl_2$, the final result will not be affected by these. So it should be alright to use these samples with this kit.

5. I wanted to ask you about your product (LDH-Cytotoxicity Assay Kit II (500 assays)). Is it suitable to be used for both human and mouse cell culture?? Also regarding measuring the absorbance is it ok if the reference wavelength is 620 nm or should it be 650 nm.

Yes, both mouse and human cells can be used with this kit. This kit is in fact compatible with all mammalian samples. The reference wavelength for detection for this assay is 450 nm, not 650 nm. You can use a slightly different wavelength than the recommended one as long as your plate reader specifications mention that range of band width. For example if the band width range is +/- 20, you can use 430 - 470 nm wavelength.

6. In your opinion would the phenol red in a cell culture medium interfere with the reaction/reading of products K2027-500?

Yes, phenol red in the media can contribute to the final reading, which is why we suggest using the background control with just the cell culture media, which will account for any contribution by everything in the media including the phenol red.

7. K2027: The assay utilizing an enzymatic coupling reaction: LDH oxidizes lactate to generate NADH, which then reacts with WST to generate yellow color. The intensity of the generated color correlates directly with the cell number lysed.

Special advantages of K2027: Since WST is brighter, less amount of culture medium is required for the assay, and thus the background from serum and culture medium is significantly reduced. Using the assay, cells can be cultured in regular 10% serum containing medium, no reducing serum or special medium is required for the assay. In addition, since the WST is more stable, the reaction can be read multiple times, and can also be stopped at any time point during the reaction. LDH activity can be easily quantified by spectrophotometer or plate reader at $OD_{450\ nm}$. The kit provides all necessary reagents including LDH positive control.

8. 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.

9. 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.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

15. 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.

16. 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.

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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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