LDH Cytotoxicity Kit II

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K313
Description	Cell death or cytotoxicity is classically evaluated by the quantification of plasma membrane damage. Lactate dehydragenase (LDH) is a stable enzyme, present in all cell types, and rapidly released into the cell culture medium upon damage of the plasma membrane. LDH, therefore, is the most widely used marker in cytotoxicity study. PromoCell's *LDH Cytotoxicity Kit II* utilizes the advanced WST-1 reagent for the enzymatic coupling reaction, thus allowing a fast and more sensitive detection of LDH released from damaged cells: LDH oxidizes lactate to generate NADH, which then reacts with WST to form a water-soluble yellow-orange formazan dye. The intensity of the generated color correlates directly with the number of lysed cells. Since WST-1 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-1 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}. The kit provides all necessary reagents including an LDH positive control and takes less than 1 hour.
Quantity	500 assays

Kit Components

Component	Quantity	Cap Code
WST Substrate Mix	1 vial	Amber
LDH Assay Buffer	50 ml	NM
Cell Lysis Solution	5 ml	Clear
Stop Solution	5 ml	Blue
LDH	Lyophilized	Red

Applications / Assay Protocol

A. Preparation of Working Solutions:

Reconstitute the WST Substrate Mix in 1.1 ml ddwater for 10 minutes and mix thoroughly. The solution is stable for two month at 4°C.

Reconstitute LDH Positive Control with 100 µl of LDH Assay Buffer.

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°C.

B. LDH Cytotoxicity Assay Protocol:

1. Collect cells (adherent or suspension cells) 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. **Note:** If background signal caused by inherent LDH activity in serum is too high use the minimum serum percentage appropriate for each cell type (i.e. percentage that does not affect cell viability for the assay period).

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) LDH release varies among cell types. Therefore, you must determine the optimal cell concentration (where the difference between the High and Low Controls is at a maximum) for each cell type. The optimal cell concentration for most cell lines is between 5 x 10^3 and 2-10 x 10^4 cells/well in 200 µl of media (2.5 x 10^4–1 x 10^5 cells/ml).

b) The LDH assay can also be performed with adherent cell without detaching and collecting the cells prior to treatment with the test substance. In that case centrifuge 96-well plate with attached cells at 400 g for 5 minutes (see step 3) before transfering the clear supernatant (10 µl/well) into an optically

clear 96-well plate. Make sure that cell culture conditions (e.g. density and confluency) are - as much as possible – uniform and optimal in all wells. Ensure that equilibration time is sufficient and cells show good viability before adding any test substances.

c) Trypsin may be used to remove adherent cells from a culture surface. **Be careful when trypsinizing cells to avoid membrane damages!**

d) 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, then follow the assay protocol to determine the cell number you should use. The high control should be OD450nm ~2.0 after 30 minutes treatment with 10% Cell Lysis Solution, while the low control should be OD450nm < 0.8. The reaction time should be set at ~30 minutes.

e) Positive control (5 µl LDH) can be used to test whether all reagents are working properly to response to active LDH enzyme.

f) 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°C) 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 g for 10 minutes 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 minutes** at room temperature.

6. Measure the absorbance of all controls and samples with a plate reader equipped with 450 nm (440 nm to 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 OD450 ~2.0, while the low control should be OD450 <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.

C. Calculation of the Percentage Cytotoxicity:

$$\text{Cytotoxicity (\%)} = \frac{\text{(Test Sample - Low Control)}}{\text{(High Control - Low Control)}} \times 100$$

Trouble Shooting

Low color reaction or no color reaction at all:
- Cell concentration may be too low. Check and titrate cell concentration.
- Test substances or compounds in the assay medium may inhibit LDH activity. Please check test substances and medium for LDH inhibition. Avoid pyruvate containing culture media as pyruvate inhibits the assay reaction.

Strong color reaction also in low controls:
- Cell concentration may be too high. Check and titrate cell concentration.
- Substances or compounds in the assay medium have LDH activity. Please check test substances and medium for LDH activity. Reduce serum concentration.
- High spontaneous LDH release may be due to bad condition of the cells used in the assay. Check culture conditions: some cell lines grow poor or do not survive in serum-free media (even at short incubation times). Increase serum concentration to about 1 - 5%.

Strong color reaction but low absorbance values:
- Substances or compounds in the assay medium have LDH activity. Please check test substances and medium for LDH activity. Reduce serum concentration.
- Too high background values may result in too low absorbance values if they are substracted automatically.

Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.
Related Products	For many more Cytotoxicity Assays as well as Cell Viability/Proliferation & Necrosis/Apoptosis Assays please visit our website www.promocell.com.

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