CPT-0101

LDH Based Cell Cytotoxicity Assay (WST)

(Sufficient for 500 wells, Store at 4°C)

Background Information:

Cell death or cytotoxicity is classically evaluated by assessing the degree of plasma membrane damage. Lactate dehydrogenase (LDH), a stable enzyme, is present in all cell types, and rapidly released into the culture medium upon damage to the plasma membrane. LDH is, therefore, the most widely used marker of cytotoxicity. AkrivisBio's LDH Cytotoxicity Assay Kit utilizes WST, a tetrazolium detection reagent to give a fast, highly sensitive measure of LDH release from damaged cells. Color intensity correlates directly with the number of damaged cells. Any background from serum or culture medium is significantly reduced due to the color intensity of WST. Cells can be cultured in regular serum containing medium. No special medium is required. Due to increased stability of WST, the reaction can be read multiple times, or can be stopped at any time point during the reaction. LDH activity is quantified by plate reader at OD 450 nm. The assay takes less than 1 hour.

Assay Components:

WST Detection Reagent	lyoph	Clear NM	CPT-0101A
Assay Buffer	50 ml	NM	CPT-0101B
Cell Lysis Solution	5 ml	Amber NM	CPT-0101C
Stop Solution	5 ml	Blue NM	CPT-0101D
LDH (Positive Control)	lyoph	Red	CPT-0101E

Storage and Handling Considerations:

Store the kit at 4°C.

WST Detection Reagent: Reconstitute with 1.1 ml DI H_2O let sit undisturbed for 10 min then vortex briefly. Stable for two months at 4°C.

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

LDH Assay Mix: Add 200 µl of WST Detection Reagent to 10 ml of Assay Buffer. Don't make more than needed. LDH Reaction Mix is stable for 4-6 weeks at 4°C.

LDH-Cytotoxicity Assay Protocol:

1. Collect cells (adherent or suspension) and wash once with fresh culture medium, then add 2-10 x 10^4 cells in 100 µl to wells of a 96-well plate as follows:
   a. **Background Control:** 100 µl cell-free culture medium per well in triplicate. This allows correction for any color due to reagents or LDH background from culture medium serum. The background value has to be subtracted from all other values.
   b. **Low Control:** 100 µl cells per well in triplicate.
   c. **High Control:** 100 µl cells per well in triplicate, plus 10 µl Cell Lysis Solution to each well and mix. To correct for the difference in volume,11 µl of medium may be used in step 4 (below).
   d. **Test Samples:** 100 µl cells per well in triplicate, plus add test substances to each well and mix.

Notes:

- Trypsin or other more gentle means may be used to detach adherent cells. The number of cells used per well depends on the cell type. To optimize the assay, do a quick test using 2, 4 and 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 ~2 $OD_{450\ nm}$ after 30 min following treatment with 10% Cell Lysis Solution. The low control should be < 0.8 $OD_{450\ nm}$ at ~ 30 min.
- Positive Control (3-5 µl LDH) is used in 1-2 wells to demonstrate that all reagents are working properly in response to active LDH enzyme.
- If the test substances are not dissolved in PBS, a solvent control should be performed by addition of the same amount of solvent without other test substances.

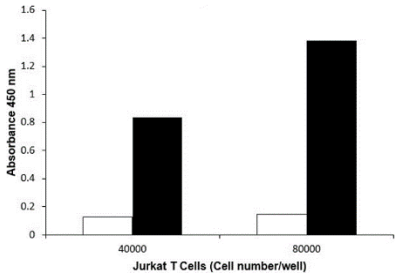
2. Incubate cells (5% CO_2, 90% humidity, 37°C) for an appropriate treatment time determined for test substance. At end of the incubation, shake the plate gently to ensure LDH is evenly distributed in the culture medium.
3. Centrifuge cells at 600 x g for 10 min to pellet the cells.
4. Transfer the clear 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 at 450 nm.

Notes:

- Adjust the reaction time to the rate of color development. The plate can be read at multiple time points until the desired reading is observed. The high control should be $OD_{450\ nm}$ ~ 2.0, while the low control should be $OD_{450\ nm}$ < 0.8.
- Stop the reaction by adding 10 µl of Stop Solution, mix and read within 48 hr. without significant changes. Protect the reaction from light and evaporation.

Calculation of Percentage Cytotoxicity:

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



LDH Cytotoxicity Assay Kit II. Jurkat cells were incubated in a 96-well plate as described in the protocol. The LDH Assay was performed using 10 µl of culture medium using the WST probe. Low Control (white bar); High control (black bar).

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