Frequently Asked Questions

LDH CYTOTOXICITY ASSAY KIT

1. How does LDH assays work?

LDH assays are designed to measure the activity of lactate dehydrogenase (LDH) enzyme released into the cell culture medium from cells that have lost the integrity of the plasma membrane. The enzyme located in the cytoplasm is released to the culture medium, being a cytotoxicity marker. LDH catalyzes the conversion of lactate to pyruvate, producing NADH. This NADH reduces a tetrazolium salt (e.g., INT or WST), resulting in a colorimetric or spectrophotometric readout proportional to amount of LDH in the sample.

2. Can LDH assay distinguish between different modes of cytotoxicity or cell death?

No. LDH assay is not specific to any particular mode of cytotoxicity or cell death. LDH can be released from damaged or stressed cells. However, it is possible to combine LDH assay with other methods such as propidium iodide staining (*TBK0554*) or annexin V binding (*TBK0508-0515*) to distinguish between necrotic, apoptotic, and other modes of cell death.

3. What are the advantages of LDH assay to measure cytotoxicity?

The advantages of LDH to measure cytotoxicity are:

- It is a relatively simple and inexpensive method for measuring cytotoxicity.
- The assay can be rapidly performed on a wide range of cell types and experimental conditions.
- Allows analysis of cytotoxicity in real-time without disturbing cells.
- Highly sensitive and can detect even small amounts of LDH release from damaged or dying cells.
- Non-radioactive and safe to use.

4. Can LDH assay be used *in vivo*?

LDH assay is typically performed *in vitro* on cell cultures or tissue homogenates. However, researchers can also adapt LDH assay for use *in vivo* by collecting blood or other bodily fluids and measuring LDH activity. Elevated levels of LDH in the blood or other bodily fluids can indicate tissue damage or cell death. This approach has been used in clinical studies to evaluate the effects of drugs on tissue damage or organ dysfunction including liver disease, myocardial infarction, and hemolytic anemia.

5. Can LDH assay be used in bacteria cytotoxicity assays?

No. LDH is specific enzyme to eukaryotic cells. Bacteria do not produce LDH enzymes. Cytotoxicity assays based on LDH activity is suitable to measure cell death or cytotoxicity in eukaryotic cells. Nevertheless, bacterial cytotoxicity can be measured using other cytotoxicity assays based on MTT and resazurin.

6. What are the applications of the LDH assay?

The LDH assay is primarily used to:

- Assess cell membrane integrity as an indicator of cytotoxicity.
- Evaluate the effects of drugs or treatments on cells.
- Quantify cell death in apoptosis or necrosis studies.

7. What are the limitations of LDH assay?

LDH assay is not specific to any particular mode of cell death, and can also be released from damaged or stressed cells. In addition, the enzyme is present in serum. For this reason, it is necessary to adjust the serum concentration (1 - 5%) in culture medium to reduce assay background.

8. What factors can influence the LDH assay results?

- Cell density: High cell numbers can produce excessive LDH.
- Incubation time: Prolonged incubation can lead to non-specific LDH release.
- Medium composition: LDH in serum-containing media can interfere with readings.

9. How is the LDH assay controlled?

The assay typically includes the following controls:

a) Positive control: Cells treated with a lysis solution to release all LDH.
b) Negative control: Untreated or healthy cells to measure background LDH release.
c) Blank control: Media without cells to account for background absorbance.

10. Is LDH enzyme the only used biomarker to measure cytotoxicity?

No. There are some identified biomarkers associated with loss of membrane integrity or modes of cell death such as mitochondrial dehydrogenases, phosphatidylserine, proteases, caspases, etc.

11. Can the LDH assay be used in combination with other assays?

Yes. LDH assay is often combined with other assays such as:

- MTT or WST1 assays for viability.
- Annexin V/PI staining to differentiate apoptosis and necrosis.
- Caspase activity assays for more detailed apoptosis analysis.

12. What are the alternatives to LDH assay?

There are several alternative assays available for measuring cell viability and cytotoxicity, including MTT assay, ATP assay, propidium iodide staining, etc. The choice of assay depends on several factors including specific experimental conditions (type of cell or tissue, mechanism of cytotoxicity being investigated, sensitivity and specificity of the assay), available resources. There is no single "best" assay for measuring cytotoxicity, and researchers may combine multiple assays to obtain a more comprehensive assessment of cell death.

Biomarker	Cellular Process	Assay Based On	Readout
Mitochondrial dehydrogenase	Mitochondrial Respiration	MTT	Colorimetric
Phosphatidylserine	Apoptosis	Annexin V	Fluorescence
ATP	Cellular Energy Status	ATP Luminiscence Assay	Luminiscence
Plasmatic Membrane	Loss Membrane Integrity	Propidium Iodide	Fluorescence
Proteases	Proteolytic Activity	Protease Assays	Fluorescence
Caspase 3 or 7	Apoptosis	Caspase Assay	Luminiscence/ Fluorescence
Caspase 9	Apoptosis	Ac-LEHD-AMC	Fluorescence
DNA Fragmentation	Apoptosis	TUNEL Assay	Fluorescence