BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Cell Injury Assay Kit (Cat #: A-101)

COMPONENTS: LDH Assay Solution- 10 ml; store at -80℃ (200 assays)

PRODUCT DESCRIPTION: Assay of the glycolytic enzyme lactate dehydrogenase (LDH) leaked into extracellular space upon cell damage or lysis is a sensitive, precise, and convenient method for the studies of cell injury and cytotoxicity reactions (J. Immunol. Methods 64:313,1983). The Cell Injury Assay Kit is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which is water-soluble and exhibits an absorption maximum at 492 nm. Since the intensity of the red color formed is proportional to the activity of extracellular LDH, which correlates with the extent of cell damage, the assay has been widely used for detection and quantification of apoptosis as well as necrosis. Each kit is sufficient for 200 assays using the 96-well format. The assay solution should be stored in aliquots at -80℃.

PROTOCOL: Thaw LDH Assay Solution and keep it on ice shielded from light. Gently agitate the solution prior to pipetting. The solution should be aliquoted for storage after the first use.

***Cell culture medium sample-** A small aliquot of the cell culture medium should be collected prior to and at several time points after the initiation of cell assault. LDH activity present in the medium represents spontaneous LDH release, which is enhanced by cell injury and cell death. Clarify medium briefly by centrifugation if necessary and store at -20℃.

Optional: Freeze (-80℃) and thaw (room temperature) the culture dish containing both cells and medium twice at the end of the experiment to induce maximal LDH release. Transfer the freeze-thawed medium to a microtube, remove cellular debris by a 3-min spin at maximal speed, and store at -20℃. The medium can be used for measurement of maximal LDH release and normalization of LDH activity.

1. Place all medium samples on ice after thawing. Add 10 µl medium to each well of a 96-well plate. Include a 10-µl control medium well for obtaining background reading.
2. Assay is initiated by adding 50 µl LDH Assay Solution to each well. Gently and briefly agitate the plate to mix contents. Allow reaction to continue for 30 min at 37°C or until a dark red color appears. DO NOT use CO_2 incubator for this step. Note that maximal LDH release sample may need to be diluted with control medium to ensure assay linearity.
3. Stop the reaction by adding 50 µl 3% acetic acid per well. Agitate the plate briefly, and measure absorbance at 492 nm using a microplate reader. Subtract control medium reading from sample reading. The intensity of $O.D._{492\ nm}$ is proportional to the LDH activity in the medium.
4. Cell injury can be estimated from % Maximal LDH release = $(O.D._{spontaneous\ LDH} \div O.D._{maximal\ LDH}) \times 100$, or simply estimated from relative LDH release if maximal LDH release sample is not available. Alternatively, follow the kinetic format described below for plasma/serum sample.

****Plasma/serum sample-** Plasma/serum samples should be clarified by centrifugation at maximal speed if necessary. These samples are assayed using a kinetic format to eliminate interference caused by non-specific reaction. Set up assay in duplicate 96-well microplates (for 10 min and 20 min reaction).

1. Place thawed samples on ice. Add 10 µl sample to each of the two 96-well microplates. Include a 10-µl dH_2O well on each plate for obtaining background reading. Reaction is initiated by adding 50 µl LDH Assay Solution to each well. Mix contents by brief gentle agitation. Cover plates, and incubate at 37°C for 10 min. Remove one plate and stop reaction by adding 50 µl 3% acetic acid per well. Measure $O.D._{492nm}$ using a microplate reader. Subtract dH_2O reading from sample reading. Remove another plate at 20 min, stop reaction by acetic acid, and measure $O.D._{492nm}$. Subtract dH_2O reading from sample reading.
2. LDH activity in Units/ml = $(O.D._{20\ min} - O.D._{10\ min}) \times 11 \times 100 \div 10\ min = (O.D._{20\ min} - O.D._{10\ min}) \times 110$

NOTE:

- The assay solution contains the organic solvent DMSO (9% v/v) and iodonitrotetrazolium violet (2 mg/ml). Avoid skin contact. Please contact us or visit our website for MSDS information.
- A 3% acetic acid solution needs to be prepared for reaction termination.