BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: LDH Assay Solution- 5 ml; store at -80°C (100 wells)

PRODUCT DESCRIPTION: Assay of the glycolytic enzyme lactate dehydrogenase (LDH) leaked into extracellular space upon cell injury or lysis is a sensitive and convenient method for detection and measurement of 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 exhibits an absorption maximum at 492 nm. The assay has been widely used for detection and quantification of apoptosis as well as necrosis. The kit is suitable for culture medium and biological fluids such as serum and plasma. Each kit is sufficient for 100 assays using the 96-well format. The assay solution is good for many years if stored in aliquots at -80ºC.

PROTOCOL: Thaw LDH Assay Solution and keep solution on ice and shielded from light. Gently agitate solution prior to first pipetting. Freeze solution immediately after use. *Do not over thaw*.

Assays:

Cell culture medium samples: Collect a small aliquot of fresh culture medium and conditioned culture medium (~50 µl). Clarify medium in a microfuge at maximal speed (~13,000 rpm) for 5 min at 4°C. Store medium supernatants at -80°C after use.

1. Thaw medium samples quickly and keep on ice. Add 10 µl fresh medium (for background) and each conditioned medium sample to a plain (uncoated) 96-well plate.

2. Reaction is initiated by addition of 50 µl LDH Assay solution to each well. Gently agitate plate for 10 sec to mix contents.

3. Incubate plate in a 37°C incubator for 20-60 min (*do not use CO_2 incubator*). Presence of LDH in the medium would cause a time-dependent increase in cherry red color.

4. Measure optical density (O.D.) at 492 nm using a plate reader. Subtract background well reading from each sample well reading to generate ΔO.D., which is proportional to membrane leakage and cell injury/death.

Serum/plasma samples: Clarify a small aliquot of serum/plasma samples (~50 µl) in a microfuge at maximal speed (~13,000 rpm) for 5 min at 4°C. Store supernatants at -80°C after use.

1. Thaw samples quickly and keep on ice. Dilute each serum/plasma sample 3-fold with dH_2O, e.g., 20 µl dH_2O + 10 µl serum/plasma. Mix diluted samples well.

2. Add 10 µl of each 3x-diluted serum/plasma sample to duplicate wells of a plain (uncoated) 96-well plate.

3. Add 50 µl dH_2O to one set of sample wells serving as control wells. Add 50 µl LDH Assay Solution to the other set of sample wells serving as reaction wells. Gently agitate plate for 10 sec to mix contents.

4. Incubate plate in a 37°C incubator for 20-60 min (*do not use CO_2 incubator*). Presence of LDH in the sample would cause a time-dependent increase in cherry red color.

5. Measure optical density (O.D.) at 492 nm using a plate reader. Subtract control well reading from reaction well reading to generate ΔO.D. for each sample, which is proportional to membrane leakage and cell injury/death.

NOTE:

- The LDH Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Avoid skin contact. Please contact us or visit the product web page for material SDS information.