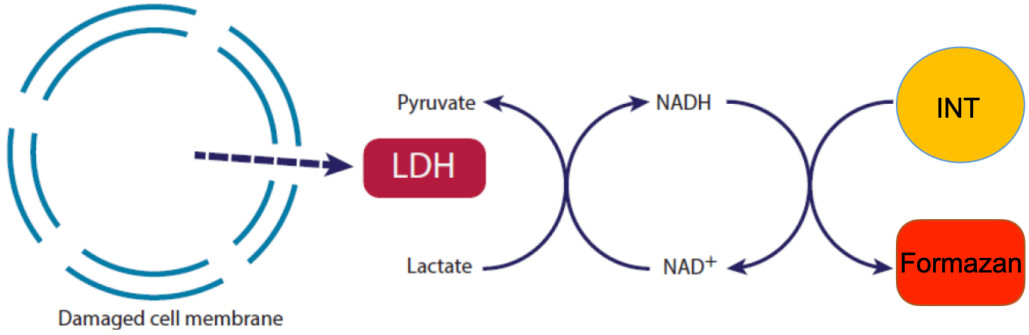
IMMUNOLOGICAL SCIENCES Product Data Sheet

IK-71001 Lactate dehydrogenase (LDH) Cytotoxicity Assay Kit

Size: 1000 Tests (10x 100 Tests)

Introduction :

Lactate dehydrogenase (LDH) Cytotoxicity Assay Kit is used to determine cytotoxicity by quantitatively measuring Lactate Dehydrogenase (LDH) activity in damaged cells. LDH is a stable cytoplasmic enzyme present in all cells. Once cells are impaired by stress, injuries, chemicals, or intercellular signals, LDH is rapidly released into the cell culture medium through the damaged plasma membrane. The cytotoxicity detection mechanism of this kit is based on the reduction of NAD+ to NADH when LDH catalyzes dehydrogenation of lactate to pyruvate. NADH further reduces a cell impermeant, water-soluble tetrazolium salt INT in the presence of an electron mediator to produce a red formazan dye. The intensity of the formazan dye thus formed is proportional to that of released LDH in the medium, which is an indication of cytotoxicity. The amount of the highly colored and soluble formazan can be measured at 490nm spectrophotometrically.



Kit Components :

Cat No	Contents of the Kit	Size	
IK-7100	LDH Cytotoxicity Assay Kit	100T	
Cat No	**Contents of the Kit**	**Package**	**Storage**
7100-1	LDH	7.5ml	-20°C avoid light 1year
7100-2	Sodium Lactate	10ml	
7100-3	Enzyme solution	2x 5ml	
7100-4	INT solution (10X)	1ml	
7100-5	INT buffer	10ml	

General Protocol :

1.Cell culture setup :

Seed cells in a 96-well culture plate in 200uL of culture medium with or without test compounds. Culture the cells in a CO2 humidified incubator at 37℃ for the desired period of time. We recommend that you prepare at least 3 replicates for each test sample. Besides test samples, three positive control cultures in medium with 1% Triton X-100 and three negative control cultures in medium without any test compounds or Triton X-100 should be included.

2. Preparation of test samples and +/- control :

Centrifuge the 96-well culture plate at 400g for 5min and transfer 150uL of supernatant from each well (test, positive and negative control wells) to the corresponding well of the 96-well test plate.

2. Preparation of the Reagents :

1) To prepare a Working Solution INT solution(1X)：Use the INT buffer to dilute INT (10X) to 1x。Such as，add 20μl INT (10X) to 180μl INT buffer. Store the Working Solution at 4℃ and protected from light. The Working Solution INT solution(1X) was freshly

prepared just before use.

2)Fresh preparation of LDH Working Solution according the following table.

Number of the test samples	1T	10T	20T	50T
Sodium Lactate	20μ l	200μ l	400μ l	1ml
INT(1X)	20μ l	200μ l	400μ l	1ml
Enzyme solution	20μ l	200μ l	400μ l	1ml
Total volume	60μ l	600μ l	1.2 ml	3ml

3) (Optional) To get the exact quantification of the LDH, customer should prepare the LDH standard by yourself(LDH standard is NOT included in the kit) , The LDH standard was freshly prepared just before use in different concentration such as 10mU/ml、5mU/ml、2.5mU/ml、1.25mU/ml、 0.65mU/ml、0mU/ml。

3. Measurements:

1) To make 60 uL of the LDH working solution for each well of 96-well plate.

2) Blending well and Incubate in the horizontal shaker for 30min at room temperature in dark (~25℃)。Read the absorbance at 490nm with plate reader.

4. Calculations:

1) Average the OD_{490nm} of replicate wells of each LDH standard, test sample, control and blank. Subtract the average OD_{490nm} value of the blank from the average OD_{490nm} values obtained with all other samples.

2) Based on the calibrated OD_{490nm} of the LDH standard, make a standard curve by plotting OD_{490nm} as a function of LDH concentration

(See Fig 1 as a typical standard curve). Determine the equation and R2 value of the trend line.

3) Suppose the equation of the trend line of the standard curve is y=Ax+B, calculate the LDH concentration of the test samples and controls as follows:

$$X=[LDH]= (OD_{490nm} -B)/A$$

4) Calculate the cytotoxicity of the test compounds as follows:

$$Cytotoxicity(\%)=\{[LDH]_{test\ samples}-[LDH]_{negative\ control}\}/\{[LDH]_{positive\ control}-[LDH]_{negative\ control}\}\times 100$$

5) If the exact concentration is not needed, the measurement of the LDH standard curve canbe skipped and the relative cytotoxicity of the test compounds can also be calculated based on the OD_{490nm} values as follows:

$$Cytotoxicity(\%)=\{[OD_{490nm}]_{test\ samples}-[OD_{490nm}]_{negative\ control}\}/\{[OD_{490nm}]_{positive\ control}-[OD_{490nm}]_{negative\ control}\}\times 100$$

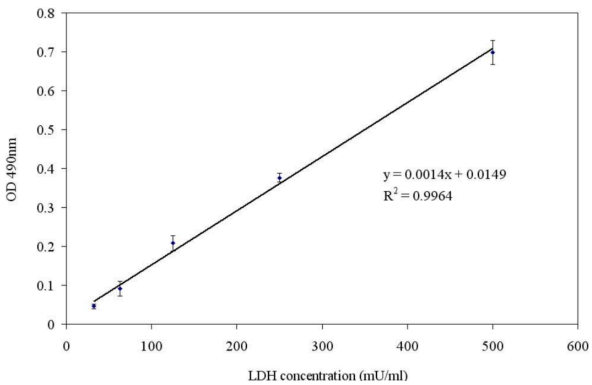


Fig1. A Typical LDH standard curve

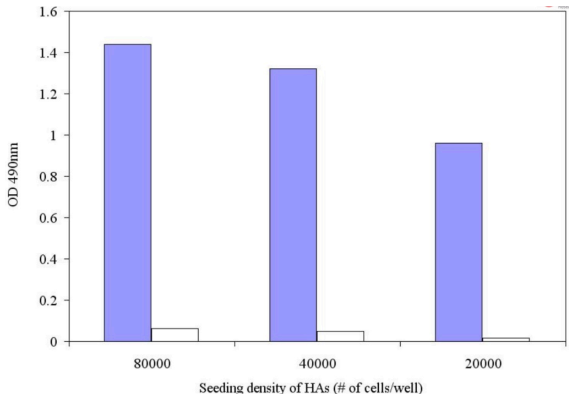


Fig.2 LDH kit is applied to Human Astrocytes(Has) cultured with (Solid bar, Positive control) and without (Open bar, Negative control) Triton X-100