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CheKine™ Lactate Dehydrogenase (LDH) Assay Kit (Dinitrobenzene Hydrazine Assay)

Cat #: KTB1118 Size: 96 T/96 S 480 T/480 S

	Lactate Dehydrogenase (LDH) Assay Kit (Dinitrobenzene Hydrazine Assay)		
REF	**Cat #:** KTB1118	LOT	**Lot #:** Refer to product label
	Detection range: 31.25-2,000 nmol/mL		**Sensitivity:** 31.25 nmol/mL
	Applicable sample: Animal and plant tissues, cells, bacteria, serum (plasma), cell culture		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Lactate Dehydrogenase (LDH, EC1.1.1.27) is widely present in animals, plants, microorganisms and cultured cells. It is the terminal enzyme of the glycolysis pathway, catalyzing the reversible reaction between pyruvate and lactic acid, accompanied by the mutual change between NAD^+/NADH. CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit (Dinitrobenzene Hydrazine Assay) can detect biological samples such as animal and plant tissues, cells, bacteria fungus, serum, plasma or cell culture. In this kit, LDH catalyzes NAD^+ oxidation of lactic acid to produce pyruvate. Pyruvate further interacts with 2,4-dinitrobenzene to produce pyruvate dinitrophenylhydrazine, which looks brown-red in alkaline solution, and the color depth is proportional to the concentration of pyruvate

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Extraction Buffer	120 mL	120 mL×5	4℃
Reagent Ⅰ	15 mL	75 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	15 mL	75 mL	4℃, protected from light
Reagent Ⅳ	40 mL	100 mL×2	4℃
Reagent Ⅴ	100 μL	500 μL	4℃, protected from light
Standard	Powder×1 vial	Powder×5 vials	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm

- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge
- Deionized water, PBS
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent II: Ready to use as supplied. Add 1.2 mL deionized water and 10 μL Reagent Ⅴ to Reagent Ⅱ for 96 T, and 6 mL deionized water and 50 μL Reagent Ⅴ to Reagent Ⅱ for 480 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃., protected from light.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅴ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Prepared before use. Add 1 mL deionized water to a Standard, dissolve thoroughly, that is 20 μmol/mL Standard. The remaining 20 μmol/mL Standard can also be stored at 4℃ and protected from light for 1 month. Using 20 μmol/mL Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	20 μL of 20 μmol/mL Standard	180	2,000
Std.2	100 μL of Std.1 (2,000 nmol/mL)	100	1,000
Std.3	100 μL of Std.2 (1,000 nmol/mL)	100	500
Std.4	200 μL of Std.3 (500 nmol/mL)	100	250
Std.5	100 μL of Std.4 (250 nmol/mL)	100	125
Std.6	100 μL of Std.5 (125 nmol/mL)	100	62.5
Std.7	100 μL of Std.6 (62.5 nmol/mL)	100	31.25
Blank	0	100	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the EP tube, wash cells or bacteria with cold PBS, centrifuge at 800 g for 2 min and discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Test Well (μL)	Control Well (μL)	Blank Well (μL)	Standard Well (μL)
Sample	10	10	0	0
Std. Standard	0	0	0	10
Deionized Water	0	10	20	10
Reagent Ⅰ	50	50	50	50
Working Reagent Ⅱ	10	0	0	0
Mix well and incubate accurately for 10 min at 37℃ (mammal) or 25℃ (other species).				
Reagent Ⅲ	50	50	50	50
Mix well and incubate accurately for 10 min at 37℃ (mammal) or 25℃ (other species). Take 100 μL reaction mix per well to a new 96-well plate or microglass cuvette:				
Reaction mix	100	100	100	100
Reagent Ⅳ	125	125	125	125

4.Mix thoroughly, incubate at 25℃ for 5 min, and measure the absorbance value at 450 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, and the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well, the Standard Well only need to be done once or twice. Every Test Well needs to be equipped with a Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 2,000 noml/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get y (nmol/mL).

2. Calculation of LDH activity:

(1) Calculated by protein concentration

Active unit definition: Catalyzing the production of 1 nmol pyruvate per minute per milligram of protein was defined as one unit of enzyme activity.

LDH (U/mg prot)=$y\times V_{Sample}\div(V_{Sample}\times Cpr)\div T\times n$**$=0.0667y\div Cpr\times n$**

(2) Calculated by sample fresh weight

Active unit definition: Catalyzing the production of 1 nmol pyruvate per minute per gram of sample was defined as one unit of enzyme activity.

LDH (U/g fresh weight)=$y\times V_{Sample}\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times n$**$=0.0667y\div W\times n$**

(3) Calculated by number of cells or bacteria

Active unit definition: Catalyzing the production of 1 nmol pyruvate per minute per 10^4 cells or bacteria was defined as one unit of enzyme activity.

LDH ($U/10^4$)=$y \times V_{Sample} \div (500 \times V_{Sample} \div V_{Total\ sample}) \div T \times n$**=0.0667y÷500×n**

(4) Calculated by volume of liquid sample

Active unit definition: Catalyzing the production of 1 nmol pyruvate per minute per milliliter of liquid was defined as one unit of enzyme activity.

LDH (U/mL)=$y \times V_{Sample} \div V_{Sample} \div T \times n$**=y×n**

V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 500: The number of cells or bacteria, in 10^4; n: sample dilution multiple; T: reaction time, 15 min.

Typical Data

The following data are for reference only, y=2852.2x-10.693, R^2=0.9997. And the experimenters need to test the samples according to their own experiments.

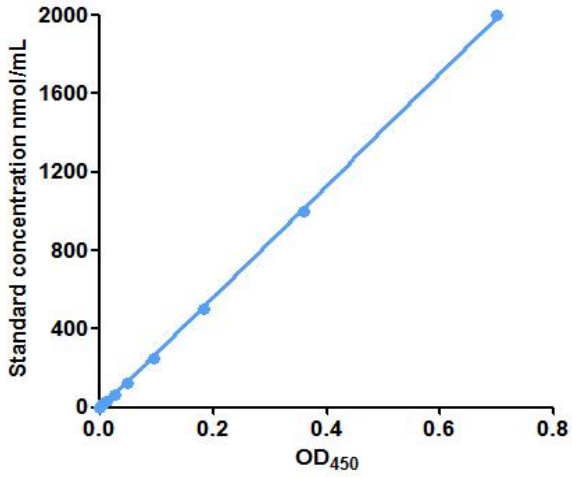

Figure 1. Standard curve.

Example-1: Take 0.1 g of arabidopsis leaf, follow the measurement steps, and use a 96-well microplate for detection. Measured $\Delta A_{Test}=A_{Test}-A_{Control}$=0.2074-0.1354=0.072. Substitute the standard curve and calculate y=194.67. Calculated according to the sample quality, LDH (U/g fresh weight)=0.0667y÷W×n=129.84 U/g.

Recommended Products

Catalog No.	Product Name
KTB1320	CheKine™ Micro Plant Soluble Sugar Assay Kit
KTB1330	CheKine™ Micro Blood Glucose Assay Kit
KTB1340	CheKine™ Micro Glycogen Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.