Lactate dehydrogenase (LDH) Colorimetric Assay Kit

Cat# CC1024 – 96T | CC1025 – 5 x 96T

Storage at 4℃ for 1 months

APPLICATION

This kit can be used to measure Lactate dehydrogenase (LDH) activity in tissues, serum (plasma), hydrothorax, culture cells, cell culture supernatants and other samples.

DETECTION SIGNIFICANCE

Lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes). LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. LDH is expressed extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease such as heart failure. Lactate dehydrogenase is composed of four subunits (tetramer). The two most common subunits are the LDH-M and LDH-H protein, encoded by the LDHA and LDHB genes, respectively. These two subunits can form five possible tetramers (isoenzymes): 4H, 4M, and the three mixed tetramers (3H1M, 2H2M, 1H3M). These five isoforms are enzymatically similar but show different tissue distribution: The major isoenzymes of skeletal muscle and liver, M4, has four muscle (M) subunits, while H4 is the main isoenzymes for heart muscle in most species, containing four heart (H) subunits.

DETECTION PRINCIPLE

Coenzyme I as a hydrogen carrier, LDH catalyzes the production of pyruvic acid from lactic acid, and pyruvic acid react with pyruvic acid to form pyruvate dinitrophenylhydrazone which is brownish red in alkaline solution. The depth of the color is proportional to the concentration of pyruvic acid. The activity of LDH can be measured indirectly by measuring the OD value at 450 nm.

KIT COMPONENTS

	Component	**Specification**	**Storage**
Reagent 1	Substrate buffer	5 mL × 1 vial	2-8℃, 3 months
Reagent 2	Coenzymel	6 mg× 1 vial	2-8℃, 3 months
Preparation of coenzyme I application solution: Dissolve a vial of powder with 1.33 mL double distilled water and it can be store at -20 for 2 weeks. It is recommended to aliquot the prepared solution and store at -20			
Reagent 3	Chromogenic agent	5 mL × 1 vial	2-8℃, 3 months, shading light
Reagent 4	Aqueous alkali	5 mL × 1 vial	2-8℃, 3 months
Preparation of Reagent 4application solution: Dilute theReagent 4 with double distilled water for 10 times. Prepare fresh solution before use.			

Reagent 5	2 μmol/mL pyruvic acid standard solution	1 mL × 1 vial	2-8℃, 3 months
Preparation of 0.2 μmol/mLpyruvic acid standard application solution: Dillute Reagent 5with double distilled water for 10 times. Prepare fresh solution before use.			

EXPERIMENTAL INSTRUMENT

Microplate, Micropipettor, Vortex mixer, Water bath, Microplate reader (450 nm)

SAMPLE PREPARATION

1. **Serum (plasma)**: Detect the sample directly. For plasma sample, heparin is recommended as an anticoagulant.
2. **Tissue samples**: Accurately weigh the tissue, add 9 times the volume of PBS (0.01 M, pH 7.4) according to the ratio of Weight (g): Volume (mL) = 1:9. Homogenize the tissue sample with homogenizer on ice. Centrifuge the homogenized tissue at 10000×g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.
3. **Cells sample**: Collect the 1×10^6 cells , add 0.3-0.5 mL of PBS (0.01 M pH 7.4). Homogenize the cells sample with homogenizer on ice . Centrifuge the homogenized cells at 10000×g for 10 min then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant .
4. **Hydrothorax sample**: Collect the fresh hydrothorax to the tubes with anticoagulant (heparin is recommended as an anticoagulant) and mix fully. Centrifuge the sample at 10000×g for 10 min, then take the supernatant and preserve it on ice for detection.

[Note]

1) Do not use hemolytic samples.

2) Oxalate should not be used as an anticoagulant because it will inhibit the activity of LDH.

3) The sample should be store at 20 or 70 if it cannot be d etected in time.

OPERATION STEPS

1. **Blank well**: add 25 μL of double distilled water.

   Standard well: add 5 μL of double distilled water and 20 μL of 0.2 μmol/mL pyruvic acid standard application solution

   Sample well: add 20 μL of Sample

   Control well: add 5 μL of double distilled water and 20 μL of sample

2. Add 25 μL of S ubstrate buffer to each well
3. Add 5 μL of Coenzyme I application solution to sample wells
4. Mix fully and incubate at 37 for 15 min.
5. Add 25 μL of Chromogenic agent to each well Mix fully and incubate at 37 for 15 min.
6. Add 250 μL of Reagent 4 application solution to each well
7. Mix fully and stand at room temperature for 5 min. Measure the OD values of each well with Microplate Reader at 450 nm

Note: The following operating table could be as a reference.

	Blank well	Standard well	Samplewell	Control well
Double distilled water (μL)	25	5		25
0.2 μmol/mL pyruvic acid standard application solution (μL)		20		
Sample (μL)			20	20
Substrate buffer (μL)	25	25	25	25
Coenzyme I application solution (μL)			5	
Mix fully and incubate at 37℃for 15 min.				
Chromogenic agent (μL)	25	25	25	25
Mix fully and incubate at 37℃for 15 min.				
Reagent 4 application solution (μL)	250	250	250	250
Mix fully and stand at room temperature for 5 min. Measure the OD value of each well at 450 nm.				

Note: (1) Do not add coenzyme I in the control well

(2) Follow the manual operatio n strictly. Do not add coenzyme I before adding substrate buffer.

REFERENCE FOR SAMPLE VOLUME

For 0.01% mouse brain tissue homogenate, 5-20 μL (determine the volume of sample according to the sample activity) For 10% human serum dilution, 10-30 μL (determine the volume of sample according to the sample activity). Dilute the sample with saline before measurement i f the LDH activity in the sample is too high.

Calculation of results

1. **For serum plasma hydrothorax cell culture supernatants sample:**

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1L of sample at 37℃ for 15 minute s in the reaction system is defined as 1 unit.

Calculation formula:

LDH activity in serum (plasma) *(U/L)*

= $(OD_{Sample}-OD_{Control})/(OD_{Standard}-OD_{Blank})$ × Concentration of standard (0.2 $\mu mol/mL$) ×1000 × the dilution multiple of sample

2. **For tissue or cells sample**:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1g protein at 37 for 15 minutes in the reaction system is defined as 1 unit

Calculation formula :

LDH activity in tissue *(U/gprot)*

=$(OD_{Sample}-OD_{Control})/(OD_{Standard}-OD_{Blank})$ × Concentration of standard (0.2 $\mu mol/mL$) ÷ Concentration of protein sample ($gprot/mL$)

Technical parameters

1. The sensitivity of the kit is 6 U/L
2. The detection range of the kit is 6-1000 U/L.
3. The intra-assay CV is 1.8 and the inter-assay CV is 2.4
4. The recovery of the kit is 98%

Notes

1. This kit is for research use only.
2. Please progress strictly with operation procedures.
3. The validity of kit is 3 months.
4. Do not use components from different batches of kit.
5. The samples should not contain decontaminant, such as SDS, Tween20, NP 40, Triton X-100 detergents
6. As the amount of sample is small, it is recommended to operate as followings:
   (1) Use left hand to assist stably hold the pipette when sampling.
   (2) Place the pipette near the bottom of the well and slowly add samples. The tip is moved upwards when adding samples to ensure that there is minimal sample residue on the tip.
   (3) If there is a scales-type centrifuge, slow centrifugation for a few minutes is recommended to ensure that the sample and reagents gather to the bottom of the well and it helps to reduce errors.
7. Do not add reagents too fast to avoid spilling out of wells.
8. Mix the wells moderately since the wells are relatively small. The mixing should not be too violently or too gently to avoid spilling or inadequate mixing. Collecting the liquid on the well wall and shaking the well.
9. There may be differences in the initial absorbance of the plate. It is recommended to measure the initial absorbance at the corresponding wavelength before use, record the difference, and then add samples to measure.
10. The amount of coenzyme I is relatively small. Therefore it is recommended to suck the pipette tips several times in the reaction solution when adding coenzyme I. Note to rep lace the used pipette tips.
11. Avoid bubbles when adding samples. Break the bubbles before measurement if there are some bubbles.

Appendix: preparation of standard curve
(This is for reference only.)

Pretreatment

Dilute 2 μmol/mL pyruvic acid standard solution with deionized water to a serial concentration. The recommended dilution gradient is as follows: 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05 μmol/mL. Then take the assay strictly according to the operation steps.

Operation table

	Blank well	Standard well
Double distilled water (μL)	25	5
Standard solution with different concentrations (μL)		20
Substrate buffer (μL)	25	25
Mix fully and incubate at 37℃ for 15 min.		
Chromogenic agent (μL)	25	25
Mix fully and incubate at 37℃ for 15 min.		
Reagent 4 application solution (μL)	250	250
Mix fully and stand at room temperature for 5 min. Measure the OD value of each well at 450 nm.		

Standard curve