D-Lactic Acid Content Assay Kit (WST colorimetry)

Note: The reagents of this product have been changed, please pay attention to and strictly follow the instructions.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC5355

Size:100T/48S

Components:

Extraction reagent I: Liquid 60 mL×1. Store at 2-8℃.

Extraction reagent II: Liquid 10 mL×1. Store at 2-8℃

Reagent I: Liquid 10 mL×1. Store at 2-8℃.

Reagent II: Powder ×1. Store at -20℃, Before use, add 160μL distilled water to dissolve. It can be stored at 2-8℃ for 4 weeks. (The reagent is a lyophilized reagent, there may be a large difference in the amount of reagent observed by the naked eye between different bottles or even a very small amount of the phenomenon, this phenomenon does not affect the use of the actual quality is the same).

Reagent II working solution: Prepare the working solution according to the ratio of reagent II (V): distilled water (V) = 10 μL: 90 μL (5T) before use;

Reagent III: Powder ×1. Store at -20℃, Dissolve with 5mL distilled water before use. After packaging, store at -20℃ to avoid repeated freezing-thawing. Store at -20℃ for 4 weeks.

Reagent IV: Liquid 4 mL×1. Store at 2-8℃.

Standard: Liquid 1 mL×1. Store at -20℃, 1000μmol/mL D-lactate acid standard solution. Before use, 20μL 1000μmol/mL D-lactic acid standard solution was mixed with 1980μL distilled water to form 10μmol/mL standard solution. Then 20μL 10μmol/mL standard solution and 620μL distilled water were mixed to form 0.3125μmol/mL standard solution for later use.

Product Description

Lactic acid is an important intermediate product in the process of biological metabolism, which is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic acid content is an important index to evaluate glycogen metabolism and aerobic metabolism. Under the action of D-lactate dehydrogenase, pyruvate is generated from D-lactic acid, and NAD^+ is reduced to produce NADH and H^+. Under the action of 1-mPMS, WST-1 reacts with NADH to produce water-soluble formazan, which has the maximum absorption peak at 450nm, based on which the content of D-lactic acid can be calculated.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/Microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, centrifuge, micro quartz cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extraction reagent I volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent I and fully homogenized on ice bath. Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL supernatant and slowly add 0.15mL Extraction reagent II, slowly blow and mix until there is no bubble. Centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
2. **Cells:** Collect cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of cells number (10^6): Extraction reagent I volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of cell amount with 1 mL of Extraction reagent I. Split the cell with ultrasonication (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, total times:3 minutes). Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL supernatant and slowly add 0.15mL Extraction reagent II, slowly blow and mix until there is no bubble. Centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
3. **Serum (plasma) or other liquids sample:** Take 100μL liquid sample and add 1mL Extraction reagent I, centrifuge at 4℃ at 12000g for 10min. Take 0.8mL supernatant and slowly add 0.15mL Extraction reagent II, slowly blow and mix until there is no bubble. Centrifuge at 4℃ at 12000g for 10min, take the supernatant on ice before testing.

II. Determination

1. Preheat the spectrophotometer or microplate reader for more than 30 minutes, adjust the wavelength to 450 nm and set spectrophotometer counter to zero with distilled water.
2. Sample Test (add Reagent in the EP tube/96 well flat-bottom plate):

Reagent（μL）	Blank tube (B)	Standard tube (S)	Test tube (T)	Control tube(C)
Sample	-	-	20	20
Standard goods	-	20	-	-
distilled water	20	-	-	20
Reagent I	90	90	90	90
Reagent II working solution	20	20	20	-
Reagent III	40	40	40	40
Reagent IV	30	30	30	30

Mix thoroughly, keep it at 37℃ for 30 minutes, read the absorbance of wavelength at 450 nm. Note the light absorption values of blank tube, standard tube, test tube and control tube as A_B, A_S, A_T and Ac respectively. Calculation $\Delta A_T = A_T - Ac$; $\Delta A_S = As - A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation of D-lactic acid content:

1. Sample weight:
   D-LA content（μmol/g weight）= $\Delta A_T \div (\Delta A_S \div C_S) \times (V_{SV}+V_{EVII}) \div (W \times V_{SV} \div V_{EVI})$
   $=0.3711 \times \Delta A_T \div \Delta A_S \div W$
2. Protein concentration:
   D-LA content（μmol/mg prot）= $\Delta A_T \div (\Delta A_S \div C_S) \times V_S \div (V_S \times Cpr) = 0.3125 \times \Delta A_T \div \Delta A_S \div Cpr$
3. Cell amount:
   D-LA content（μmol/10^6 cell）= $\Delta A_T \div (\Delta A_S \div C_S) \times (V_{SV}+V_{EVII}) \div (N \times V_{SV} \div V_{EVI})$
   $=0.3711 \times \Delta A_T \div \Delta A_S \div N$
4. Volume of liquid:
   D-LA content（μmol/mL）= $\Delta A_T \div (\Delta A_S \div C_S) \times (V_{SV}+V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI+} V_{LS})]$
   $=4.082 \times \Delta A_T \div \Delta A_S$

C_S: Standard tube concentration, 0.3125μmol/mL;
V_S: Sample volume,0.1 mL.
V_{SV}: supernatant volume,0.8 mL.
V_{EVI}: Extraction volume I, 1 mL;
V_{EVII}: Extraction volume II, 0.15 mL;
N:Number of cells, in millions.
V_{LS}: Liquid sample volume,0.1 mL.

Note:

1. Extraction reagent I contains a protein precipitator, so the supernatant cannot be used for protein concentration determination. For determination of protein content, separate tissue should be taken.
2. The determination range of ΔA is between 0.01 - 1. If the absorbance value exceeds the linear range, the sample can be diluted with distilled water and then measured again. If the absorbance value is less than the linear range, the sample size needs to be increased and then measured again. Pay attention to the synchronous calculation formula.

Experimental examples:

1. Take 0.104g of rabbit muscle was added with 1mL extraction reagent I, homogenized in ice bath, centrifuged, 0.8mL supernatant was added with 0.15mL extraction reagent II, centrifuged and the supernatant was taken according to the measurement procedure. After determination with 96-well plate, calculate $\Delta A_T = A_T - A_C = 0.321-0.179=0.142$. $\Delta A_S = A_S - A_B = 0.405-0.122=0.283$, To calculate:
   D-LA content（μmol/g weight）= $0.3711 \times \Delta A_T \div \Delta A_S \div W$

= 0.3711×0.142÷0.283÷0.104= 1.7904μmol/g weight

2. Take 100μL bovine serum and add 1mL extract solution I, centrifuge it, take 0.8mL supernatant and add 0.15mL extract solution II, centrifuge supernatant, and then follow the measurement procedure, After determination with 96-well plate, calculate $\Delta A_T=A_T-A_C=0.221-0.169=0.052$. $\Delta A_S=A_S-A_B=0.405-0.122=0.283$, To calculate:
D-LA content（μmol/mL）= $4.082\times\Delta A_T\div\Delta A_S$= 4.082×0.052÷0.283= 0.75μmol/mL

Related Products：

BC0740/BC0745	Hexokinase (HK) Activity Assay Kit
BC0540/BC0545	Pyruvate Kinase（PK）Assay Kit
BC0530/BC0535	Phosphofructokinase (PFK) Assay Kit
BC5280/BC5285	D-Lactate Dehydrogenase(D-LDH) Assay Kit
BC0680/BC0685	Lacate Dehydrogenase (LDH) Assay Kit
BC5340/BC5345	L-Lactic Acid(L-LA) Content Assay Kit

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