1. **The working instructions for LDH**

Collect the supernatant of each group of cells and centrifuge at 2148 g for 5 min at room temperature to obtain the supernatant.

Add reagents in sequence as shown in the table：

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Blank  tube | Standard tube | Measurement  tube | Control  tube |
| double-distilled water (μL) | 25 | 5 |  | 5 |
| 0.2 µmol/mL Pyruvic acid standard solution (μL) |  | 20 |  |  |
| samples (μL) |  |  | 20 | 20 |
| Matrix buffer (μL) | 25 | 25 | 25 | 25 |
| coenzyme (μL) |  |  | 5 |  |
| Mix well and incubate at 37 °C for 15 min | | | | |
| 2,4-Dinitrophenylhydrazine (μL) | 25 | 25 | 25 | 25 |
| Mix well and incubate at 37 °C for 15 min | | | | |
| 0.4 mol/L NaOH solution (μL) | 250 | 250 | 250 | 250 |
| Mix well and incubate at 37 °C for 15 min  Measure the absorbance at 450 nm in a microplate reader | | | | |

Calculation formula:

LDH activity in cell supernatant (U/L) = [(ODMeasurement－ODControl)/(ODStandard－ODBlank)] × 0.2 × 5 × 1000

1. **The working instructions for CK**

Collect the supernatant of each group of cells and centrifuge at 2148 g for 5 min at room temperature to obtain the supernatant.

Prepare the reagent mix in the ratio of Reagent I:Reagent II:Reagent III:Reagent IV:Reagent V = 8:2:5:10:5, ready to use.

Prepare the phosphorus fixing agent:

reagent VII:reagent VIII application solution:double distilled water:2.5M sulphuric acid = 1:1:2:1.

Add reagents in sequence as shown in the table：

|  |  |  |
| --- | --- | --- |
|  | Measurement tube | Control tube |
| samples (μL) | 20 |  |
| reagent mix (μL) | 300 | 300 |
| Mix well, and place in a 37 °C water bath for 30 min | | |
| reagent VI (μL) | 100 | 100 |
| samples (μL) |  | 20 |
| Vortex and mix, centrifuge 626 g for 10 min at room temperature, and remove supernatant for phosphorus determination | | |
| supernatant (μL) | 300 | 300 |
| phosphorus fixing agent (μL) | 2000 | 2000 |
| Mix well, and place in a 45 °C water bath for 15 min. Measure the absorbance at 660 nm in a microplate reader. | | |

Calculation formula:

CK viability (U/mL) = [7.4491 x (ODMeasurement－ODControl) 0.0716] x sample dilution

1. **The working instructions for SOD**

Prepare the color-developing agent according to the volume of reagent V:reagent VI:glacial acetic acid=3:3:2, prepare and store it at 4 °C.

Add reagents in sequence as shown in the table：

|  |  |  |
| --- | --- | --- |
|  | Measurement tube | Control tube |
| Reagent I (mL) | 0.5 | 0.5 |
| double-distilled water (mL) | 0.1 |  |
| samples (mL) |  | 0.1 |
| reagent II (mL) | 0.05 | 0.05 |
| Reagent III (mL) | 0.05 | 0.05 |
| Reagent IV (mL) | 0.05 | 0.05 |
| Vortex and mix, and place in a 37 °C water bath for 40 min | | |
| color developing agent (mL) | 1 | 1 |
| Leave at room temperature for 10 min. Measure the absorbance at 550 nm in a microplate reader. | | |

Calculation formula:

SOD activity (U/mL) = [(ODControl－ODMeasurement)/ODControl] ÷ 50% × (Vreverse total/Vsample)

1. **The working instructions for MDA**

Add reagents in sequence as shown in the table:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Blank tube | Standard tube | Measurement tube |
| 10 nM standard (mL) |  | 0.1 |  |
| anhydrous ethanol (mL) | 0.1 |  |  |
| samples (mL) |  |  | 0.1 |
| reagent I (mL) | 0.1 | 0.1 | 0.1 |
| Mix well. | | | |
| reagent II (mL) | 1.5 | 1.5 | 1.5 |
| reagent III (mL) | 1.5 | 1.5 |  |
| 50% glacial acetic acid (mL) |  |  | 1.5 |
| Vortex and mix, centrifuge at 626 x *g* for 10 min at room temperature.  Measure the absorbance at 532 nm in a microplate reader. | | | |

Calculation formula:

MDA content (nmol/mL) = [(ODMeasurement－ODControl)/(ODStandard－ODBlank)] × 10 nmol/mL

1. **The working instructions for GSH-Px**

Determinate the sample protein content by BCA.

Add reagents in sequence as shown in the table:

|  |  |  |
| --- | --- | --- |
|  | Non-enzymatic tube | Enzymatic tube |
| 1 mmol/L GSH (mL) | 0.2 | 0.2 |
| Sample supernatant (mL) |  | 0.2 |
| Place in a 37 °C water bath for 5 min | | |
| reagent I (mL) | 0.1 | 0.1 |
| Place in a 37 °C water bath for 40 min | | |
| Reagent II (mL) | 2 | 2 |
| Sample supernatant (mL) | 0.2 |  |
| Mix and centrifuge 626 x *g* for 10 min at room temperature  Take 1 ml of supernatant for color reaction | | |

Color reaction

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Blank  tube | Standard tube | Non-enzymatic tube | Enzymatic tube |
| GSH standard solvent application solution (mL) | 1 |  |  |  |
| 20 μmol/L GSH standard solution (mL) |  | 1 |  |  |
| Sample supernatant (mL) |  |  | 1 | 1 |
| reagent III (mL) | 1 | 1 | 1 | 1 |
| reagent (mL) | 0.25 | 0.25 | 0.25 | 0.25 |
| reagent (mL) | 0.05 | 0.05 | 0.05 | 0.05 |
| Vortex and mix, place at room temperature for 15 min  Measure the absorbance at 532 nm in a microplate reader | | | | |

Calculation formula:

GSH-Px enzyme activity = [(ODNon enzymatic tube －ODEnzymatic tube )/(ODStandard－ODBlank)] × 20μmol/L × 5 ÷ reaction time (5min) ÷ (sampling amount × Sample protein content)

1. **The working instructions for GSH-Px**

Determinate the sample protein content by BCA.

Add reagents in sequence as shown in the table:

|  |  |  |
| --- | --- | --- |
|  | Control tube | Measurement tube |
| Sample supernatant (mL) |  | 0.05 |
| reagent I (mL) |  | 1.0 |
| Reagent II (mL) | 0.1 | 0.1 |
| Mix well and react accurately at 37 °C for 1 min | | |
| reagent III (mL) | 1.0 | 1.0 |
| reagent IV (mL) | 0.1 | 0.1 |
| Sample supernatant (mL) | 0.05 |  |
| Measure the absorbance at 405 nm in a microplate reader | | |

Calculation formula:

CAT activity (U/mgprot)=(ODMeasuremen－ODControl) × 271 ÷ 0.05ml ÷ 60s ÷ protein concentration (mgprot/mL)