Creatine kinase (CK) assay kit

(Cat/No.: BC112 Size:50T/24S)

1. Measurement Principle (colorimetric method)

Creatine kinase (CK) catalyzes the reaction of adenosine triphosphate (ATP) and creatine to produce phosphocreatine, which is then rapidly hydrolyzed to phosphate. At this point, ATP and ATP remain stable. The addition of ammonium molybdate produces phosphomolybdic acid, which can be further reduced to molybdenum blue. The enzyme activity can be calculated based on the amount of inorganic phosphorus produced.

2. Composition and Preparation (Reagent kit shelf life: 6 months)

Reagent 1: 6mL liquid per bottle, store frozen or at 4℃.

Reagent 2: 1.5mL liquid per bottle, store frozen or at 4℃.

Reagent 3: 2 vials of powder. Add 2 mL of double-distilled water to each vial before use and store at 4℃. (**Note:** The amount of powder is small and may adhere to the tube wall or cap. It is not an empty tube. If it is not visible to the naked eye before use, centrifuge at 4000 rpm for 2 minutes before use.)

Reagent 4: One vial of powder. Dissolve each vial in 6 mL of double-distilled water before use (dissolving is slow, so you can continue to vortex to mix until dissolved). Store at 4℃.

Reagent 5: 2 vials of powder, freeze and add 2 mL of double-distilled water to each vial before use. Store at -20°C or below for 5 days after preparation.

Reagent 6: 6mL liquid per bottle, store at room temperature or 4℃.

Reagent 7: 1 bottle of powder, store at 4℃. Add 60mL of double-distilled water and heat appropriately to dissolve. The dissolved solution is **the reagent 7 working solution** . Store at 4℃.

Reagent 8: 2 bottles of powder, store at 4℃. When ready to use, add 20mL of double-distilled water to each bottle to dissolve it. This is **the working solution of Reagent 8.** Store at 4℃. Discard if the color darkens.

2.5 mol/L sulfuric acid: 50 mL × 1 bottle, store at room temperature.

double-distilled water (100mL each) are included for use in preparing reagents.

Preparation of **phosphorus determinant :** Reagent 7 working solution : Reagent 8 working solution : Double distilled water : 2.5 mol/L sulfuric acid = 1 : 1 : 2 : 1. The prepared phosphorus determinant should be light yellow. If it is colorless, it is ineffective; **if it is blue, it indicates phosphorus contamination. The phosphorus determinant should be prepared fresh before use.** After preparation, it can be stored at 4℃ for 48 hours.

3. Required Instruments and Reagents

Visible spectrophotometer and 1cm path length cuvette, vortex mixer, centrifuge, 37-45℃ water bath or incubator, beaker, protein assay reagent (for tissue or cell samples, available from our company).

4. Operation Procedure

1. Sample pretreatment:

Liquid samples such as serum (plasma): direct sampling (after preliminary testing);

Tissue samples: Accurately weigh the tissue and add 9 times the volume of physiological saline at a weight (g): volume (mL) ratio of 1:9. Homogenize mechanically under ice-water bath conditions to prepare a 10% tissue homogenate. Centrifuge at 3500 rpm for 10 minutes and collect the supernatant for testing **(pre-test the supernatant to select the optimal sampling concentration before conducting the formal experiment; in addition, the protein concentration of the supernatant must also be measured, and protein assay kits are available from our company)** .

Cell culture: After collecting cells, add 0.3 mL of physiological saline to each cell sample (the number of cells should not be less than 10^6 , the more the better). Sonicate the cells in an ice-water bath (power 200-300W, run for 5 seconds, pause for 15 seconds, repeat 3-5 times), centrifuge at 4000 rpm for 10 minutes, and collect the supernatant for testing (**pre-test the supernatant to select the optimal sampling concentration before the formal experiment; the protein concentration of the supernatant needs to be measured, and protein assay kits are available from our company**) **.**

2. Operating Procedure: (**Note** : Prepared reagents should be preheated to 37°C for 5 minutes before measurement.) (If there are many samples, a mixed reagent can **be prepared:** Reagent **1: Reagent 2: Reagent 3 application solution: Reagent 4 application solution: Reagent 5 application solution = 8:2:5:10:5** (Add 300 μL of the mixed reagent to react). Prepare only the amount needed, and prepare immediately before use. **After preparation, preheat the reagents to 37°C for 5 minutes before measurement** .)

	Measurement tube	control tube
Sample to be tested (μL)	20	
Reagent 1 (μL)	80	80
Reagent 2 (μL)	20	20
Reagent 3 working solution (μL)	50	50
Reagent 4 Application Solution (μL)	100	100
Reagent 5 Application Solution (μL)	50	50
Vortex mix and then bathe in a 37°C water bath for 20 minutes.		
Reagent 6 (μL)	100	100
Sample to be tested (μL)		20
Vortex to mix, centrifuge at 3500 r/min for 10 min, and collect the supernatant for phosphorus determination.		
Supernatant (μL)	300	300
Phosphorus fixative (μL)	2000	2000
Mix well, °incubate at 45°C for 15 minutes, zero the tube with double-distilled water, and measure the absorbance of each tube at 660nm with a 1cm optical path. Calculate the enzyme activity of CK based on the standard curve.		

[Note]: For detailed pre-testing, please refer to "Exploring the Optimal Sampling Concentration" (point 6 of this instruction manual); each sample should have one test tube and one control tube.

3. Calculation formula:

①Serum calculation formula:

$$\text{CK Activity (U/mL)} = \text{CK Activity (U/mL) calculated from the standard curve} \times N = [7.4491 \times (A_{\text{Assay}} - A_{\text{Control}}) - 0.0716] \times N$$

N: Dilution factor of the sample before testing;

② Formulas for calculating tissues or cells:

$$\text{CK Activity (U/mgprot)} = \frac{\text{CK Activity (U/mL) calculated from the standard curve}}{C_{\text{pr}}} = \frac{[7.4491 \times (A_{\text{Assay}} - A_{\text{Control}}) - 0.0716]}{C_{\text{pr}}}$$

Cpr: Protein concentration in tissue sample homogenate, mgprot/mL (prot represents protein).

(See Appendix I for the standard curve)

[Note]: The kit does not include CK standard. Users can directly apply our formula by following the operation table, but please be sure that the measured results meet the requirements of point 6 in the instructions (absolute OD value controlled between 0.05 and 0.5).

4. Calculation Example:

Example 1: 20 μL of mouse serum was used for CK detection. The absorbance of the test tube was 0.352, and the absorbance of the control tube was 0.278. The CK content was calculated based on the standard curve fitting equation:

$$\text{CK Activity (U/mL)} = 7.4491 \times (0.352 - 0.278) - 0.0716 = 0.480\ \text{U/mL}$$

Example 2: 20 μL of 2% rat liver homogenate supernatant was used for CK detection. The absorbance of the test tube was 0.386, and the absorbance of the control tube was 0.178. The protein concentration of 2% rat liver homogenate was 2.268 mg prot/mL. The calculated result is:

$$\text{CK Activity (U/mgprot)} = \frac{[7.4491 \times (0.396 - 0.178) - 0.0716]}{2.268} = 0.652\ \text{U/mgprot}$$

Example 3: 20 μL of 5% Chinese sturgeon gill homogenate supernatant was used for CK detection. The absorbance of the test tube was 0.441, and the absorbance of the control tube was 0.314. The protein concentration of the 5% Chinese sturgeon gill homogenate was 0.6429 mg prot/mL. The calculated result is:

$$\text{CK Activity (U/mgprot)} = \frac{[7.4491 \times (0.441 - 0.314) - 0.0716]}{0.6429} = 1.36\ \text{U/mgprot}$$

5. Precautions

1. All samples must be tested in both the test tube and the control tube.
2. Tissue homogenate should not be stored at 4°C for more than 10 hours. Freezing tissue blocks can extend the storage time.
3. Serum should not be stored at 4°C for more than 10 hours; freezing can extend the storage

time.

4. This method is a micro-volume, sensitive, and rapid method. Therefore, strict requirements are placed on the test tubes. The tubes must be completely free of phosphorus. If the tubes have contained H_3PO_4 buffer salts, they must be thoroughly cleaned by boiling them in detergent and water, rinsing them with tap water, and finally rinsing them with double-distilled water. **It is best to use disposable plastic tubes or new glass tubes** . This avoids phosphorus contamination. This point is very important and often the key factor in the success or failure of the experiment.
5. Once the phosphorus fixative is prepared, it should not be left for too long. Generally, it can be stored at 4℃ for 48 hours. It is best to prepare it immediately before use. Keep it in the refrigerator at all times.
6. When processing large batches of samples, it is best to use a simplified operation method, which is faster and more accurate.
7. All reagent preparation equipment must be dedicated to this purpose or be new (including pipettes for sulfuric acid, containers for double-distilled water, and pipette tips for various reagents).

6. Exploring the optimal sampling concentration

Exploring the optimal sampling concentration

Note: The optimal sampling concentration varies depending on the type of sample. Based on the CK standard curve (see Appendix), it is best to determine an optimal sampling concentration before testing each new sample. Generally, the absolute OD value (OD of the test tube minus the OD value of the control tube) should be controlled at around 0.2 (for large-scale experiments, the absolute OD value can be between 0.05 and 0.4).

1. Optimal sampling concentration for serum (plasma) :

Normally, CK activity in serum (plasma) is relatively low. However, if the CK activity in the user's model group is significantly increased, it is best to perform a preliminary test on the model group before batch experiments. If the CK activity is >3.5 U/mL, the serum from the model group needs to be diluted with physiological saline before testing. If the content is too high (the flat part of the curve), no significant difference between groups will be visible.

2. Explanation of the optimal sampling concentration for **tissue homogenate:**

If you are using this kit to test a new sample, it is best to prepare three test tubes with different concentrations first. For example, when testing muscle tissue homogenate, sample concentrations of 1%, 2%, and 5% respectively. Then calculate the enzyme concentration and select the sample concentration between 0.01 and 0.1 mg/mL (i.e., enzyme activity between 0.35 and 3.5 U/mL, where the curve is basically linear) as the optimal sampling concentration for batch experiments. If the concentration is too high (the flat part of the curve), the significant difference between groups will not be visible, and the sample concentration needs to be diluted before testing. If the concentration is too low, the experimental error will greatly affect the measurement results, and the sample concentration needs to be increased before testing.

This approach is very helpful for analyzing research results and performing t-tests.

Appendix I: Preparation of the CK Standard Curve

1. Preparation of reagents:

Take CK standard (70U/vial, not provided in this kit), dissolve and dilute with double-distilled water to prepare concentrations of 0.175, 0.350, 0.700, 1.050, 1.400, 1.750, 2.100, 2.450, 2.800, 3.150, and 3.500 U/mL. Prepare other reagents as described in the previous sample preparation.

[Note]: The prepared reagents need to be preheated at 37°C for 5 minutes before the measurement.

2. Simplified measurement operation table:

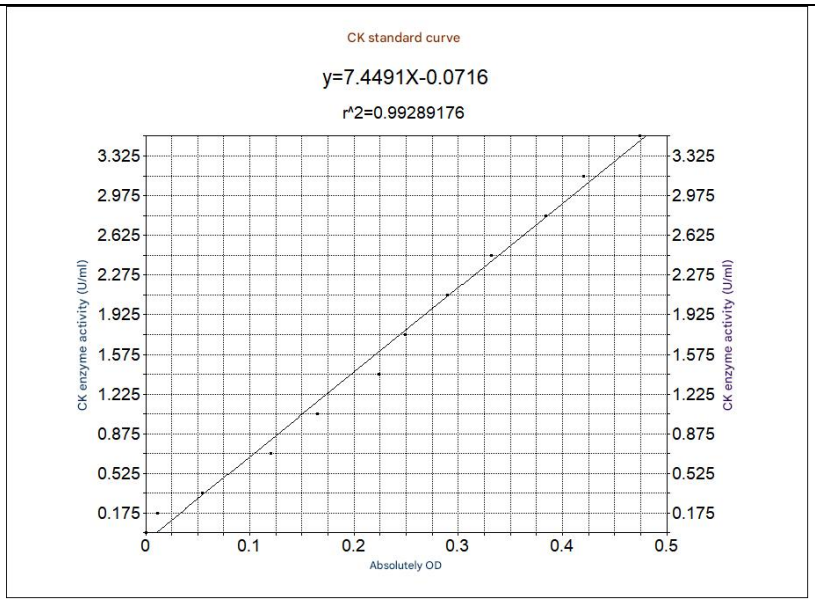
	Measurement tube	control tube
CK standard (μL)	20	
Mixed reagents (μL) (preheated to 37°C)	300	300
Vortex mix and then bathe in a 37°C water bath for 20 minutes.		
Reagent 6 (μL)	100	100
CK standard (μL)		20
Vortex to mix, centrifuge at 3500 r/min for 10 min, and collect the supernatant for phosphorus determination.		
Supernatant (μL)	300	300
Phosphorus fixative (μL)	2000	2000
Vortex mix, °bathe in a 45°C water bath for 15 minutes, zero the sample with double-distilled water, and measure the absorbance of each tube at 660nm with a 1cm optical path using a spectrophotometer.		

3. Measurement results:

CK enzyme activity (U/mL)	0.175	0.35	0.7	1.05	1.4	1.75
Compare OD values	0.276	0.276	0.283	0.288	0.288	0.289
Measure OD value	0.287	0.33	0.403	0.453	0.512	0.538
Absolute OD value	0.011	0.054	0.12	0.165	0.224	0.249

CK enzyme activity (U/mL)	2.1	2.45	2.8	3.15	3.5
Compare OD values	0.29	0.29	0.274	0.276	0.294
Measure OD value	0.58	0.622	0.658	0.698	0.768
Absolute OD value	0.29	0.332	0.384	0.422	0.474

A standard curve for the control (CK) standard was plotted with the concentration of the CK standard (U/mL) on the ordinate and the absolute OD value on the x-axis, as shown below:



The standard curve is for user reference only. Users do not need to create it; they can simply calculate it according to the formula. If you need to create it, please prepare your own standard sample.