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CreatineKinase(CK) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0336

Size:100T/96S

Components:

Extractsolution: 110 mL×1. Storage at 4℃ .

Reagent I:powder×1, stored at -20℃ and protect from light. Dissolved in 5mL of distilled water before use; the reagents that cannot be used up shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Reagent II: powder×1, storedat -20℃ .Dissolved in 0.5 mL of distilled water before use; the reagents that cannot be used up shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

ReagentIII:powder×1, storedat -20℃ . Dissolved in 0.5 mL of distilled water before use; the reagents that cannot be used up shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

ReagentIV: powder×1, storedat -20℃ .Dissolved in 0.65 mL of distilled water before use; the reagents that cannot be used up shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Reagent V： 5 mL×1, storage at 4℃ .

Product Description:

Creatine kinase (CK) (EC 2.7.3.2) is also known as creatine phosphokinase, which mainly exists in heart, muscle and brain. It can reversibly catalyze the trans-phosphoryl reaction between creatine and ATP. It is an important kinase directly related to cell energy transport, muscle contraction and ATP regeneration.

CK catalyzes creatine phosphate and ADP to generate creatine and ATP, hexokinase catalyzes ATP and glucose to generate glucose-6-phosphate, and glucose-6-phosphate dehydrogenase catalyzes glucose-6-phosphate and $NADP^+$ to generate NADPH, resulting in an increase of 340 nm light absorption value, which is used to express CK enzyme activity.

Reagents and Equipment Required but Not Provided

Scales, low temperature centrifuge, constant temperature water bath, spectrophotometer/microplate reader, micro quartz cuvette/96 wellflat-bottom(UV) plate and distilled water.

Procedure

I. Extraction of crude enzyme solution:

1. Tissue sample:

The proportion of tissue mass (g): volume of Extract solution (mL): 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of Extract solution) for ice bath homogenate. Centrifuge at 10000×g for 15 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Serum sample:

Direct determination.

3. Cell sample:

The number of cells (10^4): the volume of the Extract solution(mL) is 500- 1000:1 (1 mL of Extract solution is recommended to be added to 5 million cells), the Extract solution is added, and the cells are broken by ultrasonic wave in ice bath (Power: 300W, ultrasonic: 3s, interval: 7s, total time: 3 minutes).Then centrifuged at 10000×g for 10 minutes at 4℃,take the supernatant and place it on ice for testing.

II. Test procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm, and adjust to zero with distilled water.

2. Working solution: mix Reagent I, Reagent II, Reagent III, Reagent IV and Reagent V in the proportion of 70:4:7:10:90 (volume ratio) before use. Prepare when the solution will be used. Incubate for 20 minutes at the room temperature before use(this step cannot be omitted).

3. Operation table: add the following reagents into 1 mL cuvette

Reagent Name (μL)	Blank Tube (A_B)	Test Tube (A_T)
crude enzyme solution		40
Working solution	90	90
Distilled water	110	70
Add the above reagents into the micro quartz cuvette/96 wellflat-bottom(UV) plate respectively, mix them well and measure the absorbance value A1 at 340nm for 10s, quickly place them in a 37℃ water bath/incubator for 3minutes (the temperature controlled microplate reader can be set to 37℃). Take out the absorbance value A2 at 190s and calculate the $\Delta A_T = A2_T - A1_T$, $\Delta A_B = A2_B - A1_B$, $\Delta A = \Delta A_T - \Delta A_B$. Blank tube only needs to be done 1-2 times.		

III. Calculation of CK:

1. Calculation by micro quartz cuvette

(1) Calculated by tissue protein concentration:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount ofenzymecatalyzesthe production of 1 nmolof NADPH per minute at 37℃ and pH7.0every milligram of protein.

CK activity (U/mg prot) = $\Delta A \div (\varepsilon \times d) \times V_{RT} \times 10^9 \div (V_S \times Cpr) \div T = 268 \times \Delta A \div Cpr$

(2) Calculated by the quality of tissue samples:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzyme catalyzesthe production of 1 nmolof NADPH per minute at 37℃ and pH7.0everygram of sample.

CK activity (U/g fresh weight) = $\Delta A \div (\varepsilon \times d) \times V_{RT} \times 10^9 \div (V_S \div V_{ST} \times W) \div T = 268 \times \Delta A \div W$

(3) Calculated by serum volume:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzymecatalyze the production of 1 nmolof NADPH per minute at 37℃ and pH7.0every milliliter of serum.

CK activity (U/mL) = $\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div V_S \div T = 268 \times \Delta A$

(4) By cell count:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount ofenzymecatalyze the

production of 1 nmolof NADPH per minute at 37℃ and pH7.0every 10000 cells.

CK activity (U/10^4cell)=ΔA÷(ε×d)×V_{RV} ×10^9÷(V_S ÷ V_{ST} ×N)÷T=268 ×ΔA÷ N

ε: Molar extinction coefficient of NADPH, 6.22×10^3 L/mol/cm;

d: Light diameter of cuvette, 1 cm;

V_{RT} : Total volume of reaction system, 2×10^{-4} L;

V_S : The volume of sample in reaction system, 0.04 mL;

V_{ST} : The volume of extract solution, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: The mass of sample mass, g;

N: The number of cells, 10^4 units;

T: reaction time, 3 minutes.

2. Calculated by 96 well(UV) plate

Change the d=1 cm in the above formula to 0.6 cm (96 well plate optical diameter) for calculation.

Note:

1. The CK of serum is not stable. The samples are collected and measured as soon as possible. The CK of serum is stable for 24 hours after being stored at 4℃ in dark.

2. The protein content of the sample needs to be determined separately. BCA protein content determination kit can be used for determination.

3. If the OD value is greater than 0.6, the sample can be diluted properly with the extract solution, and calculation formula can be changed according dilution ratio.

4. ΔA_B generally does not exceed 0.01.

Experimental instances:

1. Take 0. 1g of mouse brain, add 1mL of extract solution, homogenate and grind. Take the supernatant then dilute with extract 8 times and detect according to the measured steps. Calculate ΔA_T = $A2_T$-$A1_T$=0.4175-0. 1314=0.2861, ΔA_B = $A2_B$-$A1_B$ = 0. 1304-0. 1256 = 0.0048, ΔA = ΔA_T-ΔA_B = 0.2861-0.0048 = 0.2813, calculate the enzyme activity according to sample weight:

CK activity（U/g weight）=268×ΔA÷W×8（dilution ratio）=268×0.2813÷0. 1×8（dilution ratio）=6031.072 U/g weight.

2. Take 200μL serum of rabbit to detect directly, calculateΔA_T=$A2_T$-$A1_T$=0.5761-0.4649=0. 1112，ΔA_B=$A2_B$-$A1_B$=0. 1304-0. 1256=0.0048， ΔA=ΔA_T-ΔA_B=0. 1112-0.0048=0. 1064, calculate the enzyme activity according to volume of serum:

CK activity（U/mL）=268×ΔA=268×0. 1064=28.5152 U/mL.

References:

[1] Defang Li, Ning Lu, JichunHan,et al.Eriodictyol Attenuates Myocardial Ischemia-Reperfusion Injury through the Activation of JAK2. Frontiers in Immunology. January 2018;(IF3.845)

[2] Xu Y, Meng X, Hou X, et al. A mutant of the ButhusmartensiiKarsch antitumor-analgesic peptide

exhibitsreducedinhibition to hNav1. 4 and hNav1. 5 channels while retaining analgesic activity[J].Journal of Biological Chemistry, 2017, 292(44): 18270- 18280

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