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Creatine Kinase (CK) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0337

Size:50T/48S

Components:

Extract solution: 60 mL ×1. Storage at 4℃ .

Reagent I: powder×1, stored in dark at -20℃ .Dissolved in 10 mL of distilled water before use, the unused reagentshall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Reagent II: powder×1, stored at -20℃ .Dissolved in 0.5 mL of distilled water before use, the unused reagent shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Reagent III: powder×2, stored at -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, stored at -20℃ .Dissolved in 0.65 mL of distilled water before use, the unused reagent shall be stored at -20℃ after repacking, repeated freezing and thawing are prohibited.

Reagent V: 15 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, 1 mL quartz cuvette 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).Centrifuge at 10000×g for 10 minutes at 4℃, the supernatant and place it on ice for testing.

II. Test procedure

1. Preheat the spectrophotometer 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 Vin 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	-	200
Working solution	450	450
Distilled water	550	350
Add the above reagents into the 1 mL quartz cuvette respectively, mix them well and measure the absorbance value A1 at 340 nm for 10 s, quickly place them in a 37℃ water bath for 3 minutes (thetemperature controlled microplate reader can be set to 37℃), take out the absorbance value A2 at 190 s 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) Calculated by tissue protein concentration:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzyme catalyze the production of 1 nmolof NADPH per minute at 37℃ and pH7.0 every 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 catalyze the production of 1 nmolof NADPH per minute at 37℃ and pH7.0 every gram 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 enzyme catalyze the production of 1 nmolof NADPH per minute at 37℃ and pH7.0 every 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 of enzyme catalyze the production of 1 nmolof NADPH per minute at 37℃ and pH7.0 every 10000 cells.

CK activity (U/10^4cell)=$\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \div V_{ST} \times N) \div T = 268 \times \Delta A \div N$

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, 0.001 mL;
V_S: The volume of sample in reaction system, 0.2 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.

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 4 times and detect according to the measured steps. Calculate$\Delta A_T=A2_T-A1_T=0.638-0.149=0.489$, $\Delta A_B=A2_B-A1_B=0$, $\Delta A=\Delta A_T-\Delta A_B=0.489-0=0.489$, calculate the enzyme activity according to sample weight:

CK activity (U/g weight) $=268\times\Delta A\div W\times4$ (dilution ratio) $=268\times0.489\div0.1\times4$ (dilution ratio) =5242.08U/g weight.

2. Take 200μL serum of duck to detect directly, calculate$\Delta A_T=A2_T-A1_T=0.445-0.423=0.022$, $\Delta A_B=A2_B-A1_B=0$, $\Delta A=\Delta A_T-\Delta A_B=0.022-0=0.022$, calculate the enzyme activity according to volume of serum:

CK activity (U/mL) $=268\times\Delta A=268\times0.022=5.896$ 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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