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CheKine™ Micro Creatine Kinase (CK) Activity Assay Kit

Cat #: KTB1012 Size: 48 T/96 T

	Micro Creatine Kinase (CK) Activity Assay Kit		
REF	**Cat #:** KTB1012	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells, Serum		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Creatine Kinase (CK, EC 2.7.3.2) mainly exists in the heart, muscle, brain and other tissues. It can reversibly catalyze the phosphorylation reaction between creatine and ATP. It plays an important role in energy operation, muscle contraction and ATP regeneration, and it is an important indicator for clinical diagnosis of heart and brain diseases. CheKine™ Micro Creatine Kinase (CK) Activity Assay Kit provides a simple, convenient and rapid CK activity detection method, which is suitable for the detection of animal tissues, cells, serum and other samples. The principle is that CK catalyzes creatine phosphate and ADP to generate creatine and ATP, hexokinase catalyzes ATP and glucose to form 6-phosphate glucose, and 6-phosphate glucose dehydrogenase catalyzes 6-phosphate glucose and $NADP^+$ to generate NADPH, there is a s characteristic absorption peak at 340 nm. The rate of NADPH increase at 340 nm can reflect CK activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	-20℃, protected from light
Reagent Ⅱ	5 mL	10 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Ice Maker, refrigerated centrifuge, water bath
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Before use, add 5 mL deionized water for 48 T and 10 mL deionized water for 96 T to fully dissolve. The remaining reagents should be store at -20℃ and protected from light after aliquoting to avoid repeated freezing and thawing.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Solution: Before use, mix Reagent I and Reagent II at a ratio of 1:1 according to the dosage. The prepared working solution is stable at 4℃ for 7 days, please use it as soon as possible after preparation.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. Serum CK is unstable, so it should be determined as soon as possible after sample collection, and it can be stable for 24 h when stored at 4°C, protected from light. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum: Direct detection.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Working Solution for 2 min at 37°C.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Blank Well（μL）	Test Well（μL）
Sample	0	40
Deionized Water	100	60
Working Solution	100	100

4. After mixing quickly, the temperature of the microplate reader was set at 37°C. Record the absorbance values of 10 s and 1 min 10 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A_{Test}=(A_{Test2}-A_{Test1})-(A_{Blank2}-A_{Blank1})$.

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculation of CK activity in serum

Unit definition: At 37°C and pH 7.0, one enzyme activity unit defines as 1 nmol NADPH produced by each milliliter of serum per min.

CK (U/mL)= $[\Delta A_{Test} \times V_{total} \div (\varepsilon \times d) \times 10^9] \div V_{sample} \div T$**$=1,608 \times \Delta A_{Test}$**

2. Calculation of CK activity in tissue of the sample

(1) Calculation according to the protein concentration of the sample

Unit definition: At 37°C and pH 7.0, one enzyme activity unit defines as 1 nmol NADPH produced by 1 mg tissue proteins per min.

CK (U/mg prot)=$[\Delta A_{Test} \times V_{total} \div (\varepsilon \times d) \times 10^9] \div (V_{sample} \times Cpr) \div T$**$=1,608 \times \Delta A_{Test} \div Cpr$**

(2) Calculation according to the weight of the sample

Unit definition: At 37°C and pH 7.0, one enzyme activity unit defines as 1 nmol NADPH produced by 1 g tissue per min.

CK (U/g fresh weight)=$[\Delta A_{Test} \times V_{total} \div (\varepsilon \times d) \times 10^9] \div (W \div V_{extraction} \times V_{sample}) \div T$**$=1,608 \times \Delta A_{Test} \div W$**

3. Calculation of CK activity in cells

Unit definition: one enzyme activity unit defines as 1 nmol NADPH produced by 10^4 cells per min.

CK (U/10^4 cells)=$[\Delta A_{Test} \times V_{total} \div (\varepsilon \times d) \times 10^9] \div (V_{sample} \div V_{extraction} \times 500) \div T$**$=3.2 \times \Delta A_{Test}$**

Where: V_{total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L, $V_{Extraction\ Buffer}$: the volume of the Extraction Buffer, 1 mL; V_{sample}: the volume of the supernatant in the reaction system, 0.04 mL; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; Cpr: protein concentration (mg/mL); T: reaction time, 1 min; W: sample weight, g; 500: total number of cells , 5 million; 10^9: unit conversion factor, 1 mol=10^9 nmol.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.