Creatine Kinase (CK) Activity Assay Kit

Cat. No. KITG-079 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Creatine kinase (CK) is mainly present in tissues such as the heart, muscle, and brain. It reversibly catalyzes phosphoryl-group transfer between creatine and ATP and is an important kinase directly associated with cellular energy transfer, muscle contraction, ATP regeneration, and related processes. Creatine kinase catalyzes the formation of creatine and ATP from phosphocreatine and ADP. Hexokinase catalyzes the formation of glucose 6-phosphate from ATP and glucose. Glucose-6-phosphate dehydrogenase further catalyzes the dehydrogenation of glucose 6-phosphate to generate NADPH. NADPH has a characteristic absorption peak at 340 nm; therefore, creatine kinase activity can be characterized by measuring the change in absorbance at 340 nm.
Description	Method: Microanalysis method. Assay Format: UV spectrophotometer or microplate reader. Pack Size: 100 tests / 96 samples. Storage Temperature: -20°C.
Form	Kit
Applications	Quantitative determination of creatine kinase (CK) activity in tissues, bacteria or cells, serum, culture medium, and other liquid samples by monitoring the change in NADPH absorbance at 340 nm.
Notes	Instructions for Use Required Instruments and Reagents: UV spectrophotometer or microplate reader; micro quartz cuvette with a 10 mm optical path or 96-well UV plate; mortar or homogenizer; adjustable pipette or multichannel pipette; benchtop centrifuge; thermostatic water bath or incubator; and distilled water. 1. Preparation of

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Crude Enzyme Extract The sample amount and ratio may be adjusted appropriately according to preliminary experimental results. 1) Tissue Process the sample at a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath, centrifuge at 10,000 × g for 15 min at 4°C, collect the supernatant, and keep it on ice until measurement. 2) Bacteria or Cells Collect bacteria or cells in a centrifuge tube by centrifugation. Process the sample at a bacteria or cell count (10^4) to Extraction Solution volume (mL) ratio of (500-1000):1. It is recommended to add 1 mL Extraction Solution to 5 × 10^6 bacteria or cells. Disrupt the bacteria or cells by sonication in an ice bath at 300 W using 3 s sonication and 7 s intervals for a total time of 3 min. Centrifuge at 10,000 × g for 15 min at 4°C, collect the supernatant, and keep it on ice until measurement. 3) Serum, Culture Medium, and Other Liquid Samples Measure directly or after appropriate dilution. Note: Creatine kinase in serum is unstable. Measure as soon as possible after sample collection. Serum remains stable for 24 h when stored at 4°C protected from light. 2. Assay Procedure 1) Preheat the UV spectrophotometer or microplate reader for at least 30 min, set the wavelength to 340 nm, and zero with distilled water. 2) Add the following reagents sequentially to a 96-well UV plate or micro quartz cuvette: Test Group: 90 µL CK Working Solution, 70 µL distilled water, and 40 µL crude enzyme extract. Blank Group: 90 µL CK Working Solution and 110 µL distilled water; do not add crude enzyme extract. 3) Mix thoroughly and start timing immediately. Measure absorbance at 340 nm at a total elapsed time of 10 s and record the values as A_1 test and A_1 blank. 4) Incubate accurately at 37°C for 180 s. Measure absorbance at 340 nm at a total elapsed time of 190 s and record the values as A_2 test and A_2 blank. 5) Calculate ΔA test = A_2 test - A_1 test; ΔA blank = A_2 blank - A_1 blank; and ΔA = ΔA test - ΔA blank. The blank group only needs to be measured 1-2 times. 3. Creatine Kinase Activity Calculations 3.1 Calculation Formulas for Measurement with a 96-Well UV Plate 1) Based on Tissue Protein Concentration CK (U/mg prot) = [ΔA × Vreaction,total × 10^9] / [ε × d_1 × Cpr × Vsample

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× T] = 535.9 × ΔA / Cpr 2) Based on Tissue Sample Mass CK (U/g) = [ΔA × Vreaction,total × Vsample,total × 10^9] / [ε × d_1 × W × Vsample × T] = 535.9 × ΔA / W 3) Based on Bacteria or Cell Count CK (U/10^4 cells) = [ΔA × Vreaction,total × Vsample,total × 10^9] / [ε × d_1 × bacteria or cell count × Vsample × T] = 535.9 × ΔA / bacteria or cell count 4) Based on Liquid Sample Volume CK (U/mL) = [ΔA × Vreaction,total × 10^9] / [ε × d_1 × Vsample × T] = 535.9 × ΔA 3.2 Calculation Formulas for Measurement with a Micro Quartz Cuvette 1) Based on Tissue Protein Concentration CK (U/mg prot) = [ΔA × Vreaction,total × 10^9] / [ε × d_2 × Cpr × Vsample × T] = 268 × ΔA / Cpr 2) Based on Tissue Sample Mass CK (U/g) = [ΔA × Vreaction,total × Vsample,total × 10^9] / [ε × d_2 × W × Vsample × T] = 268 × ΔA / W 3) Based on Bacteria or Cell Count CK (U/10^4 cells) = [ΔA × Vreaction,total × Vsample,total × 10^9] / [ε × d_2 × bacteria or cell count × Vsample × T] = 268 × ΔA / bacteria or cell count 4) Based on Liquid Sample Volume CK (U/mL) = [ΔA × Vreaction,total × 10^9] / [ε × d_2 × Vsample × T] = 268 × ΔA Variable Definitions: Vsample: volume of crude enzyme extract added to the reaction system, 0.04 mL; Vsample,total: total volume of crude enzyme extract, 1 mL; Vreaction,total: total reaction volume, 200 μL = 2 × 10^{-4} L; ε: molar extinction coefficient of NADPH, 6.22 × 10^3 L/mol/cm; d_1: optical path length of the 96-well UV plate, 0.5 cm; d_2: optical path length of the micro quartz cuvette, 1 cm; Cpr: sample protein concentration, mg/mL; W: sample mass, g; bacteria or cell count: expressed in units of 10^4; T: reaction time, 180 s = 3 min; 10^9: unit conversion factor, 1 mol = 10^9 nmol. 4. Precautions 1) If the measured absorbance is greater than 0.6, dilute the crude enzyme extract appropriately with Extraction Solution and repeat the measurement. Modify the calculation accordingly. 2) Complete the readings accurately at 10 s and 190 s to ensure the accuracy of the experimental results. When using a 96-well UV plate, use a multichannel pipette and perform the measurements in batches to ensure consistent reaction times among groups. 3) To ensure accurate results and avoid reagent loss, carefully read the

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	instructions before measurement, confirm that reagent storage and preparation are adequate, ensure that the operating steps are clear, and pretest 2-3 samples expected to show substantial differences. If problems arise during the procedure, contact the relevant personnel promptly.
Storage	Kit Components and Storage: Extraction Solution: liquid, 110 mL × 1 bottle; store at 4°C. Reagent 1: powder × 1 bottle; store at -20°C. Before use, add 5 mL distilled water and dissolve completely. After reconstitution, aliquot and store at -20°C; avoid repeated freezing and thawing. Reagent 2: powder × 1 tube; store at -20°C. Before use, add 500 µL distilled water and dissolve completely. After reconstitution, aliquot and store at -20°C; avoid repeated freezing and thawing. Reagent 3: powder × 1 tube; store at -20°C. Before use, add 500 µL distilled water and dissolve completely. After reconstitution, aliquot and store at -20°C; avoid repeated freezing and thawing. Reagent 4: powder × 1 tube; store at -20°C. Before use, add 650 µL distilled water and dissolve completely. After reconstitution, aliquot and store at -20°C; avoid repeated freezing and thawing. Reagent 5: liquid, 5 mL × 1 bottle; store at 4°C.
Reconstitution	Reagent Preparation: 1. Reagent 1: Before use, add 5 mL distilled water and dissolve completely. The reconstituted reagent may be aliquoted and stored at -20°C. Avoid repeated freezing and thawing. 2. Reagent 2: Before use, add 500 µL distilled water and dissolve completely. The reconstituted reagent may be aliquoted and stored at -20°C. Avoid repeated freezing and thawing. 3. Reagent 3: Before use, add 500 µL distilled water and dissolve completely. The reconstituted reagent may be aliquoted and stored at -20°C. Avoid repeated freezing and thawing. 4. Reagent 4: Before use, add 650 µL distilled water and dissolve completely. The reconstituted reagent may be aliquoted and stored at -20°C. Avoid repeated freezing and thawing. 5. CK Working Solution: Prepare immediately before use by thoroughly mixing Reagent 1, Reagent 2, Reagent 3, Reagent 4, and Reagent 5 at a volume ratio of 70:4:7:10:90 according to the required amount. Allow the prepared working solution to stand at room

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	temperature for 20 min before use.
Reaction conditions	Measurement wavelength: 340 nm. Mix thoroughly and read A_1 at 10 s total elapsed time, incubate accurately at 37°C for 180 s, and read A_2 at 190 s total elapsed time.
Unit definition	At 37°C and pH 7.0: 1. One activity unit is defined as the amount of enzyme that generates 1 nmol NADPH per minute per mg of tissue protein. 2. One activity unit is defined as the amount of enzyme that generates 1 nmol NADPH per minute per g of tissue sample. 3. One activity unit is defined as the amount of enzyme that generates 1 nmol NADPH per minute per 10^4 cells. 4. One activity unit is defined as the amount of enzyme that generates 1 nmol NADPH per minute per mL of liquid sample.

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