Creatine Kinase (CK) Activity Assay Kit (Spectrophotometry)

#Cat: NB-64-97420-50T #Size: 50T

Description

Creatine Kinase (CK) (EC 2.7.3.2), also known as creatine phosphokinase, is primarily found in tissues such as the heart, skeletal muscles, and brain. It reversibly catalyzes the transfer of a phosphoryl group between creatine and ATP and is an important kinase directly involved in cellular energy metabolism, muscle contraction, and ATP regeneration.

Detection Principle

CK catalyzes the conversion of phosphocreatine and ADP into creatine and ATP. Hexokinase then catalyzes the reaction of ATP with glucose to form glucose-6-phosphate, which is subsequently converted by glucose-6-phosphate dehydrogenase into NADPH using $NADP^+$. The resulting increase in absorbance at 340 nm is used to measure CK enzyme activity.

Packing

Taking 50T/48S packing for example:

Components	Packing	Storage
NB-64-97420-ES	60 mL x 1	4 °C
NB-64-97420-A	1 vial (powder) x 1	Store at −20°C. Before use, add 10 mL of distilled water to dissolve. If any solution remains unused, aliquot and store at −20°C. Avoid repeated freeze-thaw cycles.
NB-64-97420-B	1 vial (powder) x 1	Store at −20°C. Before use, add 0.5 mL of distilled water to dissolve. If any solution remains unused, aliquot and store at −20°C. Avoid repeated freeze-thaw cycles.
NB-64-97420-C	1 vial (powder) x 2	Store at −20°C. Before use, take out one vial and add 0.5 mL of distilled water to dissolve. If not used completely, aliquot and store at −20°C. Avoid repeated freeze-thaw cycles.
NB-64-97420-D	1 vial (powder) x 2	Store at −20°C. Before use, take out one vial and add 0.65 mL of distilled water to dissolve. If not used completely, aliquot and store at −20°C. Avoid repeated freeze-thaw cycles.
NB-64-97420-E	15 mL x 1	4 °C
Working solution: Before use, mix Reagent A, Reagent B, Reagent C, Reagent D, and Reagent E at a volume ratio of 70:4:7:10:90 according to the required volume. Prepare fresh immediately before use. Incubate at room temperature for 20 min before use.		
Before the formal assay, perform a preliminary test using 2–3 samples with expectedly large differences.		

Instructions

I. Required Equipment & Materials:

Weighing balance, refrigerated centrifuge, thermostatic water bath, UV spectrophotometer, 1 cm quartz cuvette, mortar/homogenizer, and distilled water.

II. Extraction of Crude Enzyme Solution:

1. Tissue samples:

Add NB-64-97420-ES at a ratio of 1:5–10 (g:mL) relative to the tissue weight. It is recommended to weigh approximately 0.1 g of tissue and add 1 mL of NB-64-97420-ES. Homogenize the tissue in an ice bath, then centrifuge at 10,000 × g for 15 min at 4°C. Collect the supernatant and keep it on ice until measurement.

2. Serum samples

Measure directly without pretreatment.

3. Cell samples:

Add NB-64-97420-ES at a ratio of 500–1,000 cells ($\times 10^4$):1 mL of extraction solution. It is recommended to add 1 mL of NB-64-97420-ES to 5 million cells. Disrupt the cells by ultrasonication in an ice bath (300 W, 3 sec on/7 sec off, total duration 3 min). Then centrifuge at 10,000 × g for 10 min at 4°C and collect the supernatant for measurement.

III. Assay Procedure

1. Preheat the spectrophotometer/microplate reader for 30 min, set the wavelength to 340 nm, and zero with distilled water.
2. Add the following reagents to a 1 cm quartz cuvette:

	Blank Tube（μL）	Sample Tube（μL）
Crude enzyme solution		200
Working solution	450	450
H_2O	550	350

In a 1 cm quartz cuvette or a 96-well UV plate, add the above reagents separately and mix thoroughly. Measure the absorbance at 340 nm at 10 s, recorded as A1. Then immediately incubate at 37°C in a water bath or incubator for 3 min. Remove the cuvette/plate promptly, wipe dry, and measure the absorbance at 190 s, recorded as A2.

Calculate as follows:

ΔA Sample = A2 Sample − A1 Sample

ΔA Blank = A2 Blank − A1 Blank

ΔA = ΔA Sample − ΔA Blank

The blank only needs to be measured 1–2 times.

IV. Calculation of CK Activity

1. Calculation based on protein concentration

Definition of activity unit: At 37 °C and pH 7.0, one enzyme activity unit is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per milligram of protein.

CK Activity (U/mg prot) = ΔA ÷ (ε × d) × V1 × 10^9 ÷ (V2 × Cpr) ÷ T = 268 × ΔA ÷ Cpr

2. Calculation based on sample fresh weight

Definition of activity unit: At 37 °C and pH 7.0, one enzyme activity unit is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per gram of sample.

CK Activity (U/g fresh weight) = ΔA ÷ (ε × d) × V1 × 10^9 ÷ (V2 ÷ V3 × W) ÷ T = 268 × ΔA ÷ W

3. Calculation based on serum volume

Definition of activity unit: At 37 °C and pH 7.0, one enzyme activity unit is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per milliliter of serum.

CK Activity (U/mL) = ΔA ÷ (ε × d) × V1 × 10^9 ÷ V2 ÷ T = 268 × ΔA

4. Calculation based on cell number

Definition of activity unit: At 37°C and pH 7.0, one enzyme activity unit is defined as the amount of enzyme that catalyzes the production of 1 nmol NADPH per minute per 10^4 cells.

CK Activity (U/10^4 cells) = ΔA ÷ (ε × d) × V1 × 10^9 ÷ (V2 ÷ V3 × cell number) ÷ T = 268 × ΔA ÷ cell number

Note:

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

d: Optical path length of the cuvette, 1 cm;

V1: Total reaction volume, 0.001 L;

V2: Sample volume in the reaction system, 0.2 mL;

V3: Volume of NB-64-97420-ES, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

Cell number: calculated in units of 10^4 cells;

T: Reaction time, 3 min;

10^9: Unit conversion factor, 1 mol = 10^9 nmol.

Precautions

1. Serum CK is unstable. Samples should be assayed as soon as possible after collection. When stored at 4°C and protected from light, CK activity remains stable for up to 24 hours.
2. The protein content of the sample needs to be determined separately.
3. If the OD value is greater than 0.6, appropriately dilute the sample with NB-64-97420-ES and adjust the dilution factor accordingly in the calculation formula.
4. For protein quantification, it is recommended to use the BCA Protein Quantification Kit (NB-64-00010) manufactured by NeoBiotech.
5. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.