Creatine Kinase Activity Colorimetric Assay Kit (#BN00990)

(Catalog # BN00990; 100 assays; Store at -20°C)

I. Introduction:

Creatine Kinase (CK) also known as creatine phosphokinase (CPK) and ATP: creatine N-phosphotransferase is a common cellular enzyme (EC 2.7.3.2). It catalyzes the reversible conversion of creatine and ATP into ADP and phosphocreatine. CK is widely expressed in various tissues and cell types, with highest activity in striated muscles, heart tissue and brain. CK consists of two subunits: M (muscle) and B (brain), and has three isoenzymes: CK-MM (skeleton muscle), CK-MB (cardiac muscle), and CK-BB (brain). Increased CK level is associated with many diseases such as myocardial infarction, muscular dystrophy, pulmonary infarction and brain tumors. Accurate measurement of CK is crucial for early diagnosis, prediction and therapeutic strategy. In Assay Genie's Creatine Kinase Activity Colorimetric Assay kit, creatine kinase converts creatine into phosphocreatine and ADP. The generated phoshocreatine and ADP reacts with CK Enzyme Mix to form an intermediate, which reduces a colorless Probe to a colored product with strong absorbance at 450 nm. The CK Activity Assay is high-throughput adaptable, simple and sensitive. This assay kit can detect Creatine Kinase activity less than 1 mU.

$$\text{Creatine + ATP} \xrightarrow{\text{Creatine Kinase}} \text{Phosphocreatine + ADP}$$

$$\text{ADP} \xrightarrow{\text{CK Enzyme Mix}} \text{Intermediate} \xrightarrow{\text{CK Developer}} \text{Color detection } (\lambda = 450 \text{ nm})$$

II. Application:

- Measurement of Creatine Kinase activity in various samples.
- Diagnostic marker for many diseases.
- Screening new therapeutic drugs.

III. Sample Type:

- Serum & plasma.
- Animal tissues: muscle, brain, heart etc.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	BN00990	Cap Code	Part Number
CK Assay Buffer	25 ml	WM	BN00990-1
CK Substrate	1 ml	Blue	BN00990-2
ATP (Lyophilized)	1 vial	Orange	BN00990-3
CK Enzyme Mix (Lyophilized)	1 vial	Green	BN00990-4
CK Developer (Lyophilized)	1 vial	Red	BN00990-5
NADH Standard (Lyophilized)	1 vial	Yellow	BN00990-6
Positive Control (Lyophilized)	1 vial	Purple	BN00990-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **ATP:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot & store at -20°C. Use within two months.
- **CK Enzyme Mix:** Reconstitute with 220 µl CK Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Use within two months. Keep on ice while in use.
- **CK Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 50 µl CK Assay Buffer to generate 10 mM (10 nmol/µl) NADH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.
- **Positive Control:** Reconstitute with 200 µl CK Assay Buffer to generate 10 mU/µl stock and mix thoroughly. Aliquot and store at –20°C. Use within two months.

VIII. Creatine Kinase Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold CK Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (100 µg) per well. Adjust final volume to 50 µl with CK Assay Buffer. For Positive Control, add 2-10 µl of Positive Control into desired well(s). Adjust final volume to 50 µl with CK Assay Buffer.
   Notes:
   a. Small molecules such as ADP, NADH etc. in some tissue samples such as liver may generate background. To remove small molecules, we suggest using 10K spin column.
   b. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **NADH Standard Curve:** Dilute NADH Standard to 1 mM by adding 10 µl of 10 mM NADH Standard to 90 µl CK Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of 1 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of NADH Standard. Adjust volume to 50 µl/well with CK Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix
CK Assay Buffer	34 µl
CK Enzyme Mix	2 µl
CK Developer	2 µl
ATP	2 µl
CK Substrate	10 µl

Add 50 µl of the Reaction Mix to each well containing Standard, Positive Control and samples, mix well.

4. **Measurement:** Incubate for 20-40 min at 37^{0}C and measure OD_{450nm}.
Note: Incubation time depends on the Creatine Kinase activity in the samples. We recommend measuring the OD in a kinetic mode and choose two time points (T_1 & T_2) in the linear range to calculate the CK activity of the samples. The NADH Standard curve can read in Endpoint mode (i.e., at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. Calculate the Creatine Kinase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Creatine Kinase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Creatine Kinase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/ml = mU/ml

Where: **B** is the NADH amount from standard curve (nmol).
Δ**T** is the reaction time (min).
V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of Creatine Kinase is the amount of enzyme that will generate 1.0 µmol of NADH per min at pH 9.0 at 37^{0}C.

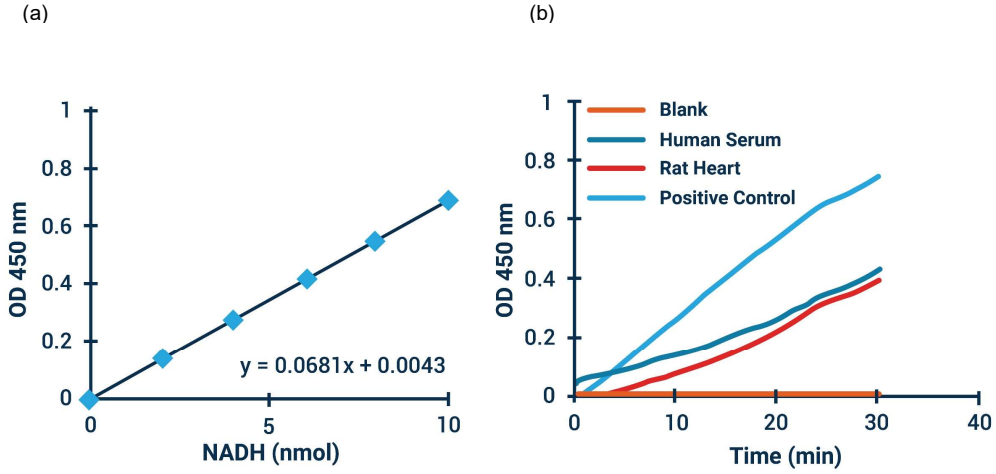

Figure 1: NADH Standard curve (a). Creatine Kinase activity in human serum (5 µl) & rat heart lysate (192 ng) (b). Assays were performed following kit protocol.

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