

Creatine Kinase (CK) Enzymatic Assay Kit

SUP6007

Enzyme Immunoassay for the determination of the Creatine Kinase in serum samples.

PRODUCT DESCRIPTION

Creatine kinase (CK) is an intracellular enzyme which catalyzes the phosphorylation of creatine using ATP. This enzyme is found primarily in the brain and in muscle tissue. The MB isozyme of the enzyme in particular is located in the heart. Following acute myocardial infarction and other heart-related trauma, serum levels of the enzyme increase significantly. Monitoring serum levels of CK enzyme has become an important means to monitor cardiotoxic side effects of therapeutic agents as well as detect the incidence of myocardial infarction.

The ID Labs Creatine Kinase (CK) Enzymatic Assay Kit is a colorimetric, plate-based assay to determine the amount of creatine kinase in serum. The kit enables biomedical researchers to detect heart damage in mice and rats. The test is based on a proven method for creatine kinase determination which utilizes a coupled enzymatic assay to specifically detect creatine kinase enzyme in fluids. It provides accurate, proven results even in complex samples. The kit contains sufficient materials to test 42 samples in duplicate and ATP product standard to verify assay performance.

The unique features of the kit are:

- High sensitivity and low detection limit (20 U/L)
- A rapid (5 minute), robust enzyme-based assay which does not require expensive Instrumentation
- High reproducibility
- Only requires 5 μ L of serum

PROCEDURE OVERVIEW

The kit measures the concentration of creatine kinase using a coupled, plate-based, colorimetric reaction. When serum is added to the reaction mix, the CK in the sample converts ADP to ATP. The ATP produced by the creatine kinase is then detected by a coupled enzymatic reaction in which the ATP is first used to produce glucose 6-phosphate which is then used by a third enzyme (glucose-6-phosphate dehydrogenase) to produce NADH from NAD⁺. NADH production is monitored by the absorbance change at 340 nm. The assay standards, included in the kit, can be used to construct a standard curve to calibrate the assay and confirm assay linearity.

KIT REAGENTS SUPPLIED

The Creatine Kinase (CK) Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit (except for the microplate) at 4 °C. The shelf life is 3 months when the kit is properly stored.

| Kit Contents | Amount | Storage |
|--------------------------|---|-------------------|
| Microtiter Plate | 1 x 96-well Plate (8 wells x 12 strips) | Room temp or 4 °C |
| CK Reagent | bottle | 4 °C |
| ATP Standard | vial | - 20 °C |
| Standard Dilution Buffer | 5 ml | - 20 °C |

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (with 340 nm absorbance filter)
Microcentrifuge
Deionized or distilled water
1.5 ml- microcentrifuge tubes
Multichannel pipet (recommended)

WARNINGS AND PRECAUTIONS

It is strongly recommended that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit

and manual. Therefore, it is important to follow the protocol coming with the kit. Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. Do not use the kit past the expiration date.

Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.

Make sure you are using only distilled deionized water since water quality is very important.

When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

ID Labs™ makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. ID Labs™ shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. For *In Vitro* Research Use Only.

SERUM DETECTION LIMIT

20 U/l

ASSAY PROCEDURE

SAMPLE PREPARATION

1. Carefully collect whole blood in a 1.5 ml microfuge tube or serum collection tube making sure to avoid hemolysis as it will release erythrocyte CK enzyme into the serum.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the “buffy coat” layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

PROTOCOL

Set up

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 340 nm.
2. Warm up kit reagents to room temperature for 30 minutes.
3. Reconstitute the Reagent Mix: Add exactly 27 ml of deionized or distilled water to the CK Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature.

IMPORTANT: The reconstituted CK Reagent can be left at room temperature for short periods (30 minutes) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4 °C (for up to 1 month). Discard the Reagent Mix 1 month after reconstitution.

Preparation of Standard Dilutions for Standard Curve

1. Label six clean microcentrifuge tubes 1, 2, 3, 4, 5 and 6 (Neg).
2. Dissolve contents of ATP Standard vial in 1.81 ml of Standard Dilution Buffer. Mix well and transfer 150 µl of dissolved Standard to Tube 1. Unused remaining portion in vial can be stored at -80°C for 6 months.
3. Serially dilute the standard by adding the appropriate volumes of dissolved ATP Standard and Standard Dilution Buffer as follows:

| Std Tube # | Preparation | Equiv Std Conc. |
|------------|--|-----------------|
| 1 | Add 150 µl of dissolved ATP Standard. | 1600 U/L |
| 2 | Add 75 µl from Standard Tube #1 + 75 µl of Standard Dilution Buffer. Mix thoroughly. | 800 U/L |
| 3 | Add 75 µl from Standard Tube #2 + 75 µl of Standard Dilution Buffer. Mix thoroughly. | 400 U/L |

| | | |
|---------|--|---------|
| 4 | Add 75 µl from Standard Tube #3 + 75 µl of Standard Dilution Buffer. Mix thoroughly. | 200 U/L |
| 5 | Add 75 µl from Standard Tube #4 + 75 µl of Standard Dilution Buffer. Mix thoroughly. | 100 U/L |
| 6 (Neg) | Add 100 µl of Standard Dilution Buffer only. | 0 U/L |

Assay Protocol

1. Add 5 µl of each sample or standard (in duplicate) to microplate wells.
2. Add 250 µl CK Reagent solution to wells containing either standard or serum.
3. Immediately measure the absorbance of each sample at 340 nm (= initial reading). After exactly 5 minutes, read the absorbance again (= 5 min reading).

Note: If the 5 min reading of a serum sample is > 1 absorbance unit, then dilute the serum 1:1 with saline and retest.

STANDARD CURVE CONSTRUCTION (Optional)

NOTE: This optional Standard Curve provides a reference for the linear range of the assay. It is simply used as a test to show that the experiment was carried out correctly; e.g. proper dilutions, temperatures, times, etc. The Standard Curve IS NOT USED to determine the concentration of CK in the samples; see **Determination of Creatine Kinase in Serum Samples below**.

A calibration curve to confirm assay linearity can be constructed using the calibration standards supplied with the kit as follows:

1. For each calibration point calculate the average corrected absorbance by subtracting the average 5 min absorbance of the “Neg” standard (0 mM ATP) from each of the average 5 min absorbance for each standard.
2. For each standard, plot the average corrected absorbance as a function of the concentration of the standard.

DETERMINEATION OF CREATINE KINASE IN SERUM SAMPLES

1. For each sample subtract the initial absorbance from the 5 min absorbance. Average these values to obtain the average absorbance increase in 5 minutes for each sample.
2. Multiply the average 5 min absorbance increase by 2,186 (conversion factor) to obtain CK activity (IU/L).

For example, if the absorbance of a sample increases by 0.3 over 5 minutes then the creatine kinase activity of the sample is:

$$0.3 \times 2186 = 655.8 \text{ IU/L.}$$