BIOGENIX
INC. PVT. LTD.

# CK-NAC

**N-acetyl-cystein- activated creatin kinase (CK-NAC) (Immunoinhibition method)**

## INTENDED USE:

CK-NAC in vitro assay for the quantitative determination of Creatine Kinase activity in human serum.

## INTRODUCTION:

Creatine Kinase (CK) is an enzyme which is found primarily in skeletal muscle, cardiac muscle and brain tissue. Elevated levels of CK are associated with myocardial infarction, various muscle disorders and diseases such as progressive Duchenne type muscular dystrophy. In myocardial infarction, peak CK levels occur 24 to 36 hours after onset of chest pain and depending on the extent of damage can reach more than 10 times normal levels.

## PRINCIPLE OF THE METHOD:

CK-NAC Reagent is a modification of the Szasz procedure. CK reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6- phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6 phosphate is oxidized by the action of the enzyme glucose-6 phosphate dehydrogenase (G-6-PDH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340 nm due to the formation of NADPH is directly proportional to the activity of the CK in the sample.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{Glucose-6-phosphatase} + \text{ADP}$$

$$\text{Glucose-6-phosphatase} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

## REAGENTS COMPOSITION:

| | |
|---|---|
| **Reagent 1** | • N-acetylcysteine (NAC) : 12 gm/L<br>• NADP : 2 gm/L<br>• DAPP : 0.050 gm/L |
| **Reagent 2** | • Imidazole : 50 gm/L<br>• Creatinine Phosphate : 45 gm/L<br>• Dextrose : 30 gm/L<br>• ADP-K : 15 gm/L<br>• G-6-PDH : 140 KU/L<br>• Hexo Kinase : 140 KU/L |

## EQUIPMENTS NEEDED BUT NOT PROVIDED

- Biochemistry Analyzer, Spectrophotometer or colorimeter measuring at 340 nm.
- Calibrated micropipettes with variable volume, range volume 5-1000 µl;
- Dry thermostat for 37± 0.1 ºC.

## REAGENT PREPARATION:

Working reagent to be prepare as 4 volume of reagent-1, mix with 1 volume of reagent-2.
This working reagent is stable for 10 day at 2-8°C

## STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C,
- Protected from light and contaminations during their use.
- Do not use reagents:
  1. After the expiration date.
  2. Signs of reagent deterioration.
  3. Presence of particles and turbidity.
  4. Working Reagent exceeds the absorbance 0.800 at 340 nm against distilled water.

## COLLECTING AND HANDLING OF SPECIMENS

- Serum and Plasma is the preferred Specimen.
- Clear Unhemolysed serum is the recommended specimen sample.
- Serum CK appears stable for 3 days at 2-8°C. It is recommended that specimens be assayed immediately after collection.

## ASSAY PROCEDURES

Being the CK-NAC all reagents to room temperature before use. Pipette in to test tube and test as fellow**:**

| Reagents | Test |
|---|---|
| Working Reagent | 1.0 ml |
| Specimen | 40 µl |

Mix well and aspirate. After the initial delay of 120 seconds, record the absorbance of the test at an interval of 60 seconds for the next 240 seconds at 340 nm. Determine the mean change in the absorbance and calculate the result.

## CALCULATION:

Activity of CK-NAC (IU/L) = ΔAbs. /min. X 4127

**SYSTEM PARAMETERS:**

| | | |
|---|---|---|
| Reaction Type (Mode) | : | Kinetic |
| Reaction Slope | : | Increasing |
| Wave Length | : | 340 nm |
| Flow Cell Temperature | : | 37 ˚C |
| Reagent volume | : | 1000 µl |
| Sample volume | : | 40 µl |
| Delay Time | : | 120 sec |
| Measuring Time | : | 120 sec |
| Factor | : | 4127 |
| Linearity | : | 1800 IU/L |
| Units | : | IU/L |
| Low Normal | : | 0 IU/L |
| High Normal | : | 325 IU/L |

**NOTES:**

1. Do not use recycled plastic tubes as they react with TBHBA chromogen leading to the false results. Always use soap and glycerol free glass tubes.
2. Contamination of reagents must be avoided. After use all the reagents must be immediately stored back at 2-8$^0$C.
3. Replug the CK-NAC reagents vial after use.
4. Contamination by soap or glycerol will affect the assay.
5. For sample values higher than 1800 IU/L, dilute the sample with normal saline and multiply the result with appropriate dilution factor.
6. As with all the diagnostic procedure, the physician should evaluate the data obtained by the use of this kit in light of other clinical information.

**QUALITY CONTROL**

To ensure adequate quality control, it is recommended that each run includes assayed normal and abnormal controls. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**REFERENCE VALUES:**

| Sample Type (Serum) | At 37˚C |
|---|---|
| Men | < 190 IU/L |
| Women | < 165 IU/L |
| Children (above 12 month) | < 225 IU/L |
| Babies | < 325 IU/L |

These values are for guidance purpose; each laboratory should establish its own reference range, according to its own geographic area.

**PERFORMANCE CHARACTERISTICS**

**Measuring range (Linearity):**

The assay is linear up to 1800 IU/L

If the results obtained were greater than 1800 IU/L, dilute the sample to 1/2 with NaCl 9 gm/L and multiply the result by 2.

**SENSITIVITY:**

Lower detection limit 1 IU/L

**ACCURACY:**

Results obtained using the reagent compared well with other commercial reagents.

**PRECISION:**

| N= 20 | Intra Run | | Inter Run | |
|---|---|---|---|---|
| | Control sample 1 | Control sample 2 | Control sample 1 | Control sample 2 |
| **Mean** (IU/L) | 79 | 1316 | 179 | 377 |
| **SD** | 1.0 | 12.2 | 2.4 | 15.2 |
| **CV%** | 1.3 | 0.9 | 1.3 | 4.0 |

The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES:**

No interference for mentioned concentrations:

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