May 17, 2019

Yale - Creatine Kinase Activity

DOI
dx.doi.org/10.17504/protocols.io.y3sfyne

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DOI: https://dx.doi.org/10.17504/protocols.io.y3sfyne

External link: https://mmpc.org/shared/document.aspx?id=219&docType=Protocol

Protocol Citation: John Stack, Gary Cline 2019. Yale - Creatine Kinase Activity. **protocols.io https://dx.doi.org/10.17504/protocols.io.y3sfyne**

Protocol status: Working
We use this protocol and it's working

Created: March 12, 2019

Last Modified: May 17, 2019

Protocol Integer ID: 21330

Keywords: Creatine Kinase Activity, creatine kinase activity summary, creatine kinase activity, creatine kinase activity in blood, creatine kinase, reactions of creatine kinase, rate of nadph formation, nadph formation, glucose

Abstract

Summary:

Procedure used to determine the creatine kinase activity in blood , serum, and plasma. Creatine kinase activity is measured by the enzymatically coupled reactions of creatine kinase, hexokinase, and glucose-6-P dehydrogenase. The rate of NADPH formation is monitored by the change in absorbance at 340 nm.

Materials

MATERIALS

- CK NADP Imidazole Reagent **Cliniqa Catalog #**R85191
- CK NADP Imidazole Buffer **Cliniqa Catalog #**R85191
- Assayed Control Serum 1 **Prolabs(cliniqa) Catalog #**R83082
- Assayed Control Serum 2 **Prolabs(cliniqa) Catalog #**R83083

Reagent Preparation:

CK NADP Imidazole Reagent: Add the appropriate volume (26mL) of CK NADP Imidazole Buffer to the powdered reagent. Gently invert reagent bottle to stir contents and allow 15 minutes for contents to mix.

CK NADP Imidazole Buffer: As supplied by vendor.

Assayed Control Serum 1: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Assayed Control Serum 2: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Troubleshooting

Before start

Analysis by automated system Cobas Mira Plus

1 Calibrate Cobas for the measurement of creatine kinase activity analysis by running two control serum.

2 Sample handling as performed by the Cobas Mira Plus.
a) Pipette 4.5 µL of sample into a cuvette slot.
b) Add 175 µL of CK NADP Imidazole Reagent.
c) Mixture is incubated at 37ºC and spun for 10 minutes.
d) Absorbance is measured at 340nm.