PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

IDTox™ Creatinine Enzymatic Assay Kit

SUP6012

Enzyme Immunoassay for the determination of the creatinine in serum samples.

PRODUCT DESCRIPTION

The ID Labs Creatinine Enzymatic Assay Kit is a plate-based colorimetric enzymatic assay for the determination of the creatinine in serum samples. Creatinine is a waste product formed throughout the body and removed from circulation through the kidneys. Creatinine levels in the blood are related to excretion rate by the kidneys. Creatinine levels in blood increase when the kidneys are diseased or damaged. For this reason elevated serum creatinine levels are a highly specific marker for kidney disease.

The kit uses a spectrophotometric, kinetic assay to detect changes in creatinine levels directly from serum samples. The features of the kit are:

- High sensitivity and low detection limit (0.5 mg /dl)
- A rapid (15 minutes) and robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility

PROCEDURE OVERVIEW

The Creatinine Enzymatic Assay Kit is a simple, direct and automation-compatible method for measuring creatinine levels in the serum. This kit uses a direct colorimetric reaction scheme. At high pH, creatinine specifically reacts with picrate reagent to form a red-colored complex. The absorbance measured at 510 nm, is proportional to the concentration of creatinine in the sample. The kit also comes with a control solution containing a creatinine standard (20 mg/dl) which can be used to calibrate the assay and generate a standard curve.

The kit contains sufficient materials to rapidly test 42 serum samples in duplicate.

After preparing the sera, the assay is performed by adding Reagent Mix into microplate wells containing 10 µl sera. After a brief incubation, the absorbance of each well at 510 nm is then measured using a plate reader. The concentration of creatinine in each sample is then directly determined from the 510 nm absorbance.

KIT REAGENTS SUPPLIED

The Creatinine Enzymatic Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit also contains enough material to construct four standard curves. Store the kit at 4°C. The shelf life of the kit, when properly stored is noted on the label.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	RT
Creatinine Color Reagent	20 ml	RT
Creatinine Buffer Reagent	20 ml	RT
Creatinine Standard (20 g/dl)	0.75 ml	2-8°C

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (510 nm)
Heater, incubator or water bath to heat samples to 37°C
Centrifuge to prepare serum samples
Deionized or distilled water (dH_2O)
PBS (phosphate buffer saline, pH 7.3) or normal saline (0.9%)
1.5 ml microfuge tubes
Multichannel pipet or repeating pipettor (recommended but not required)

ID Labs™ inc
Biotechnology
PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

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.

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.

ASSAY PROCEDURE

PREPARATION OF REAGENTS

Serum

1. Carefully prepare at least 20 µl of sera using standard production procedure (if determinations are performed in singlet then 10 µl is sufficient). Avoid hemolysis as it may release erythrocyte creatinine into the serum.

2. Creatinine in serum is reported stable for 2 days at 4°C and 3 months when frozen and properly protected against evaporation.

Note:
Samples with values above 20 mg/dl should be diluted with PBS or normal saline and re-tested. (Multiply results by dilution factor).

ASSAY PROCEDURE

Set up

Reagent Preparation

Combine equal amounts of Creatinine Color Reagent and Creatinine Buffer Reagent to a clean conical tube. Use the following table to calculate the required volume for a working reaction mix. If precipitate is visible in bottles then warm reagents to 37°C and mix to dissolve before combining reagents.

Reaction Mix Composition (per reaction)		Volume per Reaction (total)	24 Reactions	96 Reactions
Color Reagent	Buffer Reagent			
150 µl	150 µl	300 µl	7.2 ml	28.8 ml

Preparation of Creatinine Control Dilutions for Standard Curve

1. Label 6 microfuge tube: 1, 2, 3, 4, 5, 6.
2. Dilute the Creatinine Standard using water (dH2O) as described in the table below. After dilution, briefly mix each tube before performing the next dilution.

Tube #	Creatinine Standard	dH2O	Creatinine Concentration
1	100 µl	0 µl	20 mg/dl
2	50 µl	50 µl	10 mg/dl
3	25 µl	75 µl	5 mg/dl
4	12.5 µl	87.5 µl	2.5 mg/dl
5	5 µl	95 µl	1 mg/dl
6 (Neg)	0 µl	100 µ.	0 mg/dl

3. 10 µl of each diluted standard is used per reaction.

PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com

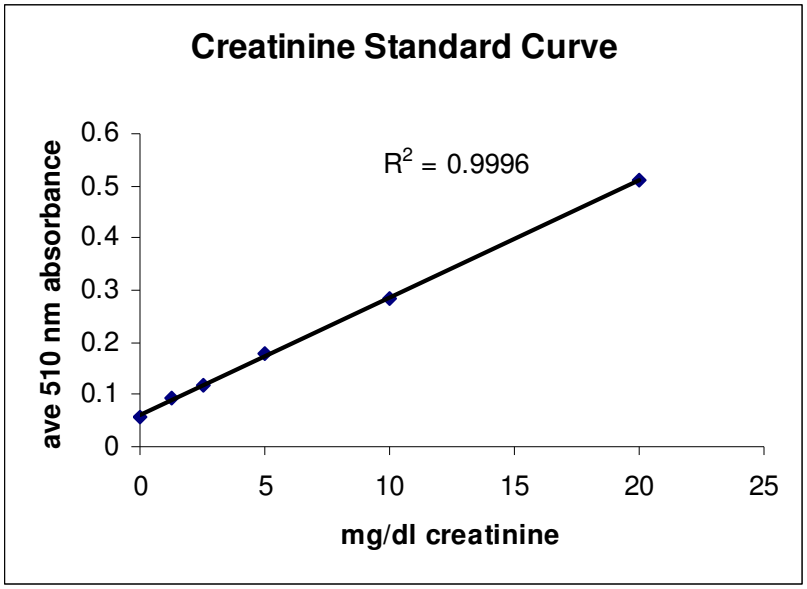
SAMPLE TEST PROCEDURE

1. Add 10 µl of each sample or standard (in duplicate) to the microplate wells.
2. Add 300 µl of reagent mix to the wells.
3. Carefully cover wells with adhesive cover sheet and incubate at 37°C for 15 minutes.
4. Carefully remove adhesive cover sheet and measure the absorbance of each sample at 510 nm.

CALCULATION OF RESULTS

Standard Curve Construction

There is a linear relationship between the concentration of creatinine in the sample and the absorbance at 510 nm. Therefore, a standard curve used to calculate the creatinine concentration in sera samples can be constructed by plotting the mean absorbance values for each of the diluted creatinine standards as a function of creatinine concentration. The standard curve provides a reference for the linear range of the assay.



`