Cr (Creatinine) colorimetric Assay Kit

Catalogue No.: EU3134

Size: 48T/96T

Reactivity: Universal

Range: 25-800μmol/L

Sensitivity: 10μmol/L

Application: For quantitative detection of creatinine in serum, plasma and urine.

Storage: 2-8°C

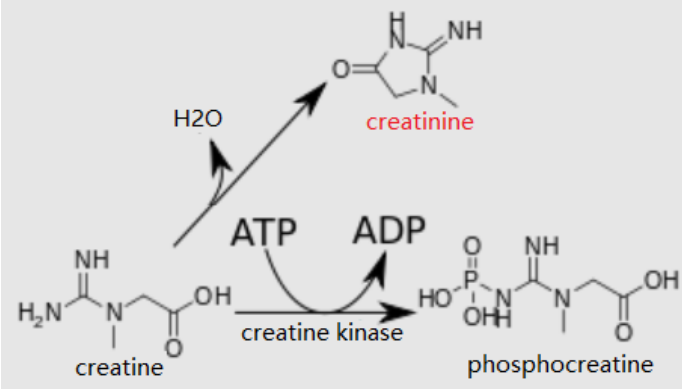
Expiry Date: see kit label

Principle: Enzyme catalysis

NOTE: FOR RESEARCH USE ONLY.

1,BACKROUND

Creatinine is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for highenergy phosphate that can be utilized by tissues for the production of ATP. Creatine either comes from the diet or is synthesized from the amino acids arginine, glycine, and methionine. This occurs in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist. Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, which diffuses into the blood and is excreted by the kidneys. In vivo, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH. Creatinine forms spontaneously from pcreatine, and under normal conditions, its formation occurs at a relatively constant rate. Intra-individual variation of creatinine levels is <15% from day to day, making it a useful marker for normalizing levels of other molecules found in urine. Altered creatinine levels may be associated with conditions that result in decreased renal blood flow, such as diabetes and cardiovascular disease.



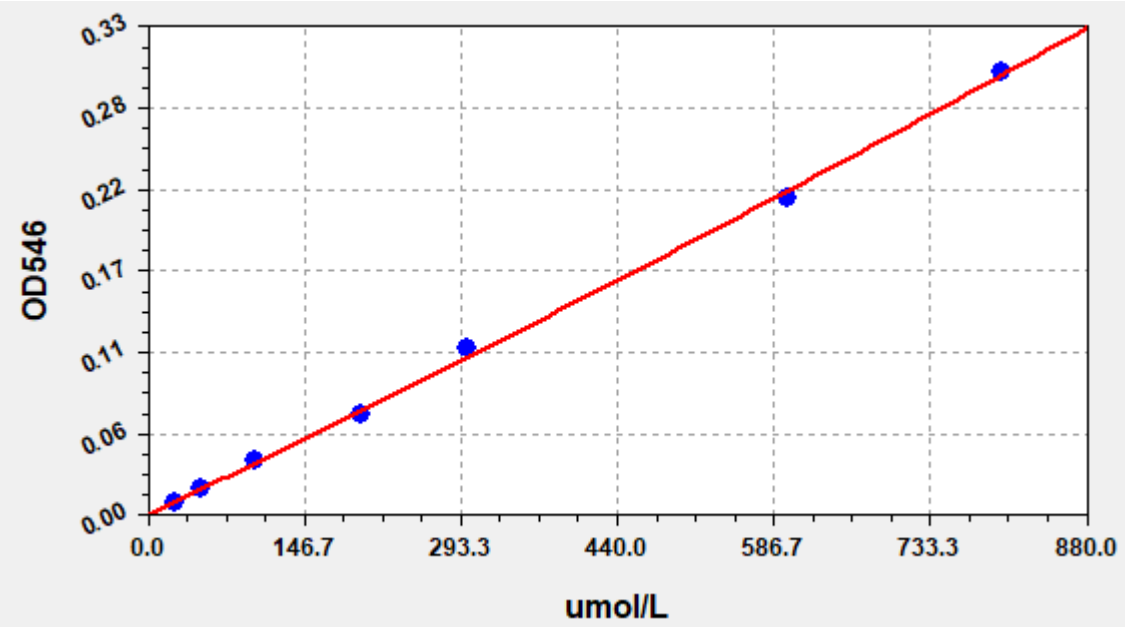
Kit Components

No.	Item	Specifications (48T/96T)	Storage
E001	ELISA Microplate	8×6/8×12	
E002	Lyophilized Standard(1000nmol/vial)	1vial/2vial	2-8°C/-20°C
E003	Enzyme Solution A	12ml/25ml	2-8°C (Avoid Direct Light)
E004	Enzyme Solution B	4ml/8ml	2-8°C (Avoid Direct Light)
E006	Plate Sealer	1/2pieces	
E007	Product Description	1copy	

Typical Data & Standard Curve

Results of a typical standard operation of a creatinine are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (OD546)

STD.(umol/L)	OD-A2	OD-A1	OD-(A2-A1)	Corrected
0	0.038	0.036	0.002	0
25	0.051	0.04	0.011	0.009
50	0.058	0.038	0.02	0.018
100	0.079	0.039	0.04	0.038
200	0.111	0.04	0.071	0.069
300	0.155	0.039	0.116	0.114
600	0.257	0.039	0.218	0.216
800	0.343	0.039	0.304	0.302



Specificity & anti-interference

This assay has high sensitivity and excellent specificity for detection of creatinine. No significant cross-reactivity or interference between creatinine and analogues was observed.

Vitamin C ≤500mg/L , Hemoglobin ≤1g/L , Cchylechyle ≤0.30%,

Bilirubin ≤342umol/L(Interference < 10%)

Recovery

Matrices listed below were spiked with certain level of creatinine and the recovery rates were calculated by comparing the measured value to the expected amount of creatinine in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	91-102	95
EDTA Plasma(n=5)	86-98	95
Heparin Plasma(n=5)	92-100	96
urine(n=5)	95-103	97

Linear range

Correlation coefficient(20-2000)umol/L,R≥0.990

Deviation from linearity(20-70)umol/L, AD≤7umol/L ， (70-2000)umol/L, AD≤10umol/L ，

Precision

Intra-Assay: CV≤6% ,Inter-Assay: CV≤4%

Stability

The kit is stored at 2-8°C (Avoid Direct Light), and not be frozen or thawed. The product is valid for 6 months. After opening, store at 2-8°C, it can be stable for 30 days, avoid contamination.

Sample data (human serum：60-120umol/L,)

Operation Procedure

Principle of the Assay

Creatinine is catalyzed by creatininase to form creatine, and creatine is catalyzed by creatinase to form sarcosine and urea, then sarcosine catalyzed by sarcosine oxidase to form glycine, formaldehyde and hydrogen peroxide. Hydrogen peroxide and chromogenic agent react under the catalysis of peroxidase to produce pink compound The creatinine content was calculated by ready the OD value at 546 nm(dominant wavelength)/700 nm (Deputy wavelength).

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
3. Storage all reagents avoid light.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't reuse tips and tubes to avoid cross contamination.
6. Avoid using the reagents from different batches together.

Material Required but Not Supplied

1. Microplate reader (wavelength:546nm/700nm)
2. 37°C incubator
3. Vortex mixer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water.

Sample Collection and Storage (universal)

- **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Urine:** Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination.

Sample Dilution

The user should estimate the concentration of the creatinine in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with distilled water, and several trials may be necessary. The test sample must be well mixed with distilled water. And also standard curves and sample should be making in pre-experiment.

Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

1, Sample prepare:

Sample	dilution ratio
Serum(human, mouse, rat, pig, dog, goat)	undiluted
plasma(human, mouse, rat, pig, dog, goat)	undiluted
urine(human, mouse, rat)	1/20 ~ 1/30 dilute

2，Standard prepare:

Diluted standard solution is unstable and must be used within 2 hours.

1, Add 1 ml distilled water into Standard tube (labeled as stock), keep the tube at room temperature for 10 minutes and mix them thoroughly.

2, Refer to the table to prepare the standard.

Standard #	concentration (umol/L)	stock (1000umol/L) (ul)	H_2O (ul)	final volume(ul)
1	800	80	20	100
2	600	60	40	
3	300	30	70	
4	200	20	80	
5	100	10	90	
6	50	5	95	
7	25	2.5	97.5	
blank	0	0	100	

The following two assay procedures are provided, please choose by yourself, the results are all for reference.

Assay Procedure(Standard operating procedure)

1. Set standard, test samples control (blank) wells on the plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.

2. **Prepare Standards:** Aliquot 10µl of standard (1-7) and blank into the standard wells.
3. **Add Samples:** Add 10µl of properly diluted sample into sample wells.
4. **Add Enzyme Solution A:** Add 210µl of enzyme Solution A into each well and mix the well.
5. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
6. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(mark A1)
7. **Add Enzyme Solution B:** Add 70µl of enzyme Solution B into each well and mix the well.
8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
9. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(mark A2)
10. **Calculation:** Use the software for calculation

    Regarding calculation, (the relative O.D.546) = (the O.D.546 A2 of each well) – (the O.D.546 A1 of each well)). The standard curve can be plotted as the relative O.D.546 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as **Curve Expert 1.3 or 1.4.(Linear Fit y=a + bx,** （Operate Video：https://www.fn-test.com/videos/elisa-sample-concentration-calculation/）**)**

Assay Procedure(Simple operating procedure)

1. Set standard (Stock), test samples, control (blank) wells on the plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. **Prepare Standard:** Aliquot 10µl of Stock Standard and blank into the standard/blank wells.
3. **Add Samples:** Add 10µl of properly diluted sample into test sample wells.
4. **Add Enzyme Solution A:** Add 210µl of enzyme Solution A into each test well mix the well.
5. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
6. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(A1)
7. **Add Enzyme Solution B:** Add 70µl of enzyme Solution B into each test well mix the well.
8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
9. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(A2)
10. **Calculation:**

$$\text{Crea(umol/L)} = \frac{\text{Sample(A2-A1)}}{\text{Stock Standard(A2-A1)}} \times \text{Stock Standard concentration}$$

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.