Code No.KK135

For research use only

Urinary Creatinine ELISA Kit

Creatinine is a metabolic waste product formed due to non-enzymatic conversion of creatine and phosphocreatine, the most part of which are found in muscle tissues. Creatinine diffuses into the blood and is excreted by kidneys into the urine. In normal condition, the excretion of creatinine is a relatively constant (1-2% of total creatine pool per day). The amount of creatinine produced is proportional to an individual's muscle mass. The urinary creatinine levels are commonly used as tool of normalization for other molecules in the urine or other tests. In addition, determination of urinary creatinine is also useful for detecting muscular and kidney disease, and estimation the extent of impairment renal function.

Our kit is convenient to quantify amount of urinary creatinine by using ELISA method. This kit is only for research use, not for diagnosis.

- Highly sensitive and specific
- Strip type well, antigen pre-coated microplate
- Assay range: 0.625－20mg/dl

Metabolizm of Creatinine

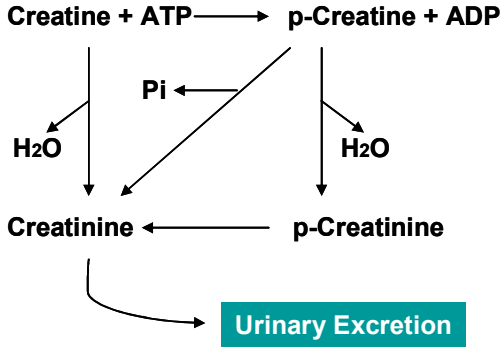
In the kidneys

Arginine + Glycine ⟶ Ornithine + Guanidoacetic acid

In the liver

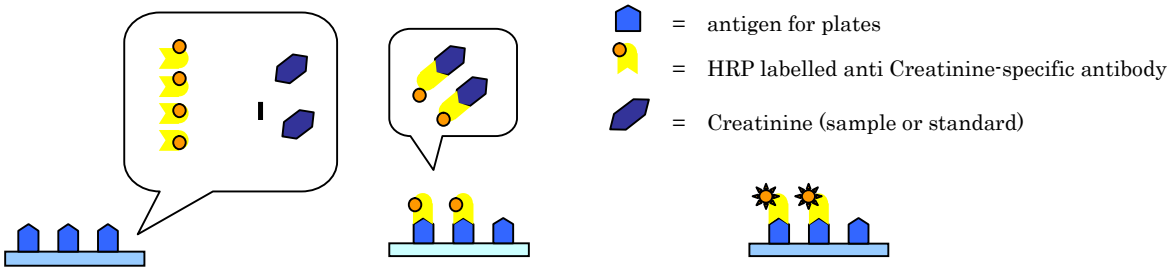
S-adenosyl-L-methionine + Guanidoacetic acid ⟶ S-adenosyl-L-methionine + Creatine

In muscle



〔**measurement principle**〕

1. Incubate with sample.



〔**Kit Contents**〕

(1)	Antigen coated microtiter plate,96 wells	1 plate
(2)	Creatinine standard	250 μl × 2
(3)	Antibody diluent	20mL × 1
(4)	HRP- anti Creatinine antibody concentrate(×100)	60 μl × 1
(5)	OPD (o-phenylendiamine) tablets	2 tab.
(6)	Substrate solution	30 mL × 1
(7)	Stop solution	15 mL × 1
(8)	Wash buffer concentrate (×20)	30 mL × 1
(9)	Dilution plate	1 plate

〔**Equipments to be supplied by the user**〕

(1) A microplate reader
(2) A micropipet
(3) A microplate washer

〔**Assay Method**〕

(1) Preparation of working solution

① Wash solution
Make sure that wash buffer concentrate does not contain any crystallized material prior to use. Working solution is prepared by dilution 30 mL of wash buffer concentrate with 570 mL of distilled deionized water. For convenience this solution can be kept at 2-8℃ up t o 14 days.

② Creatinine Standard
Prepare 6 standards by serial dilution of Creatinine standard concentrate (20mg/dl) as followings

	10	5	2.5	1.25	0.625	(mg/dl)
Standard solution 20mg/dl (μL)	100	100	100	100	100	
Deionized water (μL)	100	100	100	100	100	

③ HRP-anti Creatinine antibody (×100)
Dilute 40 μl of Anti Creatinine antibody concentrate (×100) with 4 mL of Dilution solution for 96 well reaction. Diluted antibody should not be stored.

④ Coloring solution
Add one OPD tablet to 13 mL of Substrate buffer to reconstitute the coloring solution just before use. This solution should not be stored.

(2) Preparation of urine sample
① Collect urine in sampling tube on demand.
② After centrifugation at 1500rpm for 5min, dilute the resulted supernatant 20 times with distilled deionized water.
③ Measure the amount of creatinine in remaining diluted supernatant for compensation.
※Prepared urine sample should be kept below -80℃ if necessary.

(3) Assay procedure
① Pre-reaction
Prepare standard control wells containing 70µL of anti Creatinine antibody solution and 70µL of 6 standards (20,10,5,2.5,1.25,0.625 mg/dl) in dilution plate. Likewise prepare experimental wells containing 70µL of anti Creatinine antibody solution and 70µL of prepared urinary sample in the same plate. After settlement, incubate at room temperature for 30 minutes.
*Above reaction volumes can be applied for double measurements of primary reaction. If single measurement, reduce to 40µL of each solution.

② Preparation of reaction plate
②-1 Add wash solution 300 µl to each well and wait another 30 minutes.
②-2 Discard the wash solution from the wells completely and wash with 300 µl wash solution.
Repeat this step another 2 times

③ Primary reaction
③-1 Apply 50 µl/well × 2 (In the case of measuring double wells) pre-reaction solution(See ①) and incubate for 1 hour.
③-2 After the incubation, discard the reaction solution and wash with 300 µl wash solution. Repeat this step another 2 times.

④ Coloring
Apply 100 µl Coloring solution to each well and incubate for 10 minutes at room temperature.

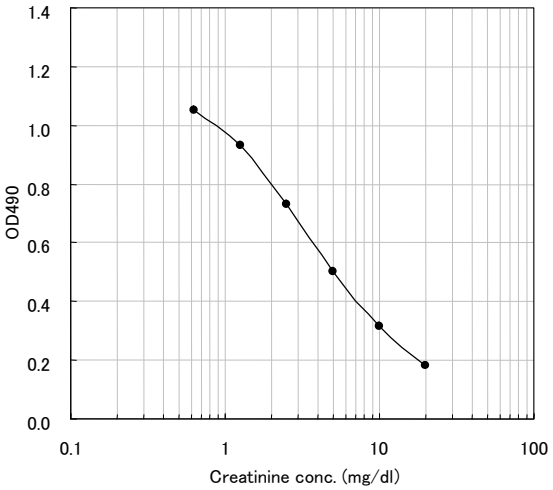
⑤ Stop reaction
Apply 100 µl of Stop solution to stop the enzymatic reaction

⑥ Read absorbance
Read absorbance of 490nm or 492 nm with a microplate reader.

⑦ Measure concentration
Measure the Creatinine concentration using standard curve.

＊ If actual measurements of sample exceed over 20mg/dl, dilute those urine samples again as possible as to evaluate within the range of 0.625 – 20mg/dl
＊ Concentration of Creatinine needs to be calculated from actual measurements by consideration of dilution ratio.

〔Standard curve〕



〔**Reproducibility**〕

Domain of standard curve :0.625－20mg/dl
Minimum measurement range for detection: 1.25mg/dl
Minimum dilution number of urine sample : ×20
Minimum sensitivity for detection: 25mg/dl
Within-run (n=15, 2 concentration): CV (%) = 8.40, 6.50
Between-run (n=10, 2 concentration): CV (%) = 8.04, 7.41
Recovery test: In the recovery study, recoveries between 92% and 100% were obtained for 20, 40 times dilutions of the sample urine
Coexistence substance : No influence to Hemoglobin 4500mg/dL・Bilirubin 180mg/dL・Glucose 1000mg/dL・Ascorbic acid 500mg/dL

〔**Usage notes**〕

① The Reagents should be stored at recommended temperature, -30℃.
② Do not use the reagents which is expired the date of usage.
③ Urine sample should be diluted more than 20 times with Dilution solution.
④ Do not leave the standard and Antibody for long time under room temperature.
⑤ The glassware for making coloring solution should be clean.
⑥ Since OPD (o-phenylendiamine) is harmful, handle with care.
⑦ Since Stop solution, 1N H_2SO_4, is strong acid, handle with care.
⑧ The kit is constructed with well-adjusted combination in each lot. Replaced combination among different lots may cause unexpected results.
⑨ This kit is only for research use. Do not use for medicinal or any other purposes.
⑩ When using the reagents, take care to avoid them from touching to skin, mucous membrane, clothes, and getting into eye.
⑪ If the reagents happen to get into eye or mouth, wash out them and consult a doctor if you need.
⑫ After using the kit, wash your hand very carefully.
⑬ If you find that the packages of the reagents are broken or something wrong, do not use them.
⑭ When you store the reagents, make sure to avoid them from vaporizing, falling down.

⑮ After using the reagents, the packages should be discarded under the established rule.
⑯ We do not guarantee the quality of the packages and accompaniments if not used according this direction.

〔**Storage**〕
All reagents: -30℃

〔References〕

1.	Iyengar MR, Coleman DW, Butler TM. Phosphocreatinine, a high-energy phosphate in muscle, spontaneously forms phosphocreatine and creatinine under physiological conditions. J Biol Chem. 1985 Jun 25;260(12):7562-7.
2.	Furter R, Kaldis P, Furter-Graves EM, Schnyder T, Eppenberger HM, Wallimann T. Expression of active octameric chicken cardiac mitochondrial creatine kinase in Escherichia coli. Biochem J. 1992 Dec 15;288 (Pt 3):771-5.
3.	Wang ZM, Gallagher D, Nelson ME, Matthews DE, Heymsfield SB. Total-body skeletal muscle mass: evaluation of 24-h urinary creatinine excretion by computerized axial tomography. Am J Clin Nutr. 1996 Jun;63(6):863-9.
4.	Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev. 2000 Jul;80(3):1107-213. Review.
5.	Miller RC, Brindle E, Holman DJ, Shofer J, Klein NA, Soules MR, O'Connor KA. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. Clin Chem. 2004 May;50(5):924-32. Epub 2004 Mar 11.

GANP

GENTAUR

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, BELGIUM
Tel 0032 16 58 90 45 | Fax 0032 16 50 90 45
www.gentaur-worldwide.com
info@gentaur.com