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 a 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

**Metabolism 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**

Creatine + ATP → p-Creatine + ADP

\[ \text{H}_2\text{O} \]

\[ \text{H}_2\text{O} \]

Creatinine ← p-Creatinine

Urinary Excretion
1. Incubate with sample.

2. Remove unbound substances

3. Detect enzymatic reaction.

**[Kit Contents]**

1. Antigen coated microtiter plate, 96 wells
2. Creatinine standard, 250 μl x 2
3. Antibody diluent, 20 mL x 1
4. HRP-anti Creatinine antibody concentrate (x 100), 60 μl x 1
5. OPD (o-phenylenediamine) tablets, 2 tab.
6. Substrate solution, 30 mL x 1
7. Stop solution, 15 mL x 1
8. Wash buffer concentrate (x 20), 30 mL x 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°C up to 14 days.

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

<table>
<thead>
<tr>
<th>Standard solution</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1.25</th>
<th>0.625</th>
</tr>
</thead>
<tbody>
<tr>
<td>20mg/dl (μL)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Deionized water</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

   ③ HRP-anti Creatinine antibody (x 100)
   Dilute 40 μl of Anti Creatinine antibody concentrate (x 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°C if necessary.

(3) Assay procedure
   ① Pre-reaction
      Prepare standard control wells containing 70μL of anti Creatinine antibody solution and 70μL of 6 standards (20,10,5,2.5,1,0.625 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.
[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-phenylenediamine) is harmful, handle with care.
⑦ Since Stop solution, 1N H₂SO₄ 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.
After using the reagents, the packages should be discarded under the established rule.

We do not guarantee the quality of the packages and accompaniments if not used according this direction.

[Storage]
All reagents: -30°C


This product is generated from GANP® mice.

Supplier

SCETI K.K.
3-6-7 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, JAPAN
Tel +81(3) 5510-2347   Fax +81(3) 5510-0133
URL: http://www.sceti.jp/export/   e-mail: exp-pet@sceti.co.jp