Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Creatinine (Mouse) ELISA Kit

Rev 06/21

(Catalog # E4369-100, 100 assays, Store at 4°C)

I. Introduction:

Creatinine is a breakdown product of creatine phosphate in muscle. Serum creatinine (a blood measurement) is an important indicator of renal health while it measure byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine, and ATP. **BioVision's Creatinine ELISA kit** is a Competitive ELISA assay for the quantitative measurement of Creatinine in mouse serum, plasma and, tissue homogenates and other biological fluids. The microtiter plate provided in this kit has been pre-coated with the target antigen. During the reaction, the target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to target. The unbound sample or excess conjugate are washed from the plate. HRP-Streptavidin (SABC) is added and incubated. Then TMB substrate solution is added to each well. The HRP enzymatic reaction is detected using TMB-substrate. Finally, an acidic stop solution terminates the enzymatic reaction. The color developed is measure at 450 nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Creatinine in mouse samples.
Detection Range: 1.2-80 nmol/ml
Sensitivity: < 0.75 nmol/ml
Assay Precision: Intra-Assay: CV < 8%; Inter-Assay: CV < 10% (CV (%) = SD/mean X 100)
Cross Reactivity: No significant cross-reactivity or interference between this analyte and its analogues was observed.

III. Sample Type:

Mouse serum, plasma, tissue homogenates and other biological fluids.

IV. Kit Contents:

Components	E4369-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4369-100-1
Lyophilized Standard (80 nmol)	2 vials	E4369-100-2
Sample/Standard dilution buffer	20 ml	E4369-100-3
Biotin-labelled detection antibody (Concentrated) (Avoid light)	60 µl	E4369-100-4
Antibody dilution buffer	10 ml	E4369-100-5
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µl	E4369-100-6
SABC dilution buffer	10 ml	E4369-100-7
TMB substrate (Avoid light)	10 ml	E4369-100-8
Stop Solution	10 ml	E4369-100-9
Wash buffer (25X)	30 ml	E4369-100-10
Plate sealers	5	E4369-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

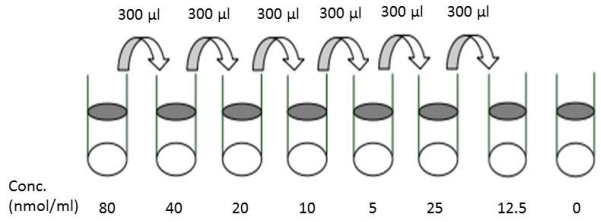
VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

4. **Standard Preparation:**

- Reconstitute the lyophilized Creatinine standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 80 nmol/ml standard stock solution. Use within 2 hours after reconstituting.
- Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
- Prepare 0.6 ml of 40 nmol/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 80, 40, 20, 10, 5, 2.5, 1.25, 0 nmol/ml



5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Serum**: Coagulate the serum for 2 hour at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma**: Collect plasma using EDTA-Na_2 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.
- **Tissue homogenates**: Rinse the tissues with ice-cold PBS (0.01 M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to be added into the PBS). Homogenize with a glass homogenizer on ice. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate**: Commercial RIPA kits are recommended. Follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate for 2x106 cells, DNA must be removed. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

VIII. Assay Protocol:

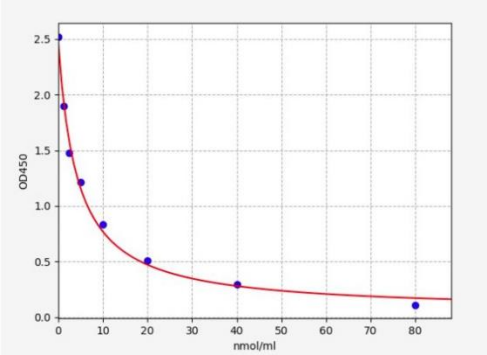
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 50 µl of each **standards** or **samples** into appropriate wells. Immediately add 50 µl of **Biotin-detection antibody working solution** to each well and cover the plate with plate sealer.
4. Gently tap the plate and incubate at 37ºC for 45 min.
5. Discard the solution and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
6. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
7. Discard the solution and wash 5 times with **1X Wash Solution** as step 6.
8. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
9. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

IX. CALCULATION:

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).** The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse Creatinine concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



STD (nmol/ml)	OD-1	OD-2	Average
0	2.485	2.557	2.521
1.25	1.872	1.926	1.899
2.5	1.453	1.495	1.474
5	1.194	1.228	1.211
10	0.818	0.842	0.83
20	0.5	0.514	0.507
40	0.29	0.298	0.294
80	0.106	0.11	0.108

X. RECOVERY:

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	89-101	95
EDTA Plasma(n=5)	85-101	91
Heparin Plasma(n=5)	88-104	95

XI. LINEARITY:

Sample	1:2	1:4	1:8
Serum(n=5)	86-102%	86-103%	85-105%
EDTA Plasma(n=5)	82-95%	85-101%	84-99%
Heparin Plasma(n=5)	84-100%	88-98%	80-97%

XII. RELATED PRODUCTS:

- Creatinine (Human) ELISA Kit (Cat. No. E4368)
- Creatinine (Rat) ELISA Kit (Cat. No. E4370)
- Creatinine Colorimetric/Fluorometric Assay Kit (Cat No. K625)
- Albumin-to-Creatinine Ratio (ACR) Assay Kit (Cat. No. K551)
- L-Carnitine Colorimetric/Fluorometric Assay Kit (Cat. No. K642)