biosensis® Creatinine (Urinary) Colorimetric Assay Kit

Catalogue Number: CRE-001-1P/5P/10P

For the quantitative determination of creatinine in human urine only if used as directed.

Please refer to the Sample Preparation Section for specific use instructions for urine.

For research use only, not for use in clinical and diagnostic procedures.

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1. Intended Use

The purpose of this kit is the quantitative determination of creatinine in human urine only if used as directed. The Creatinine (Urinary) Colorimetric Assay is not recommended for plasma or serum samples. Precipitation may occur in the wells upon the addition of the acid solution. Biosensis does not assume responsibility if this kit is used for unintended purposes. Please see our sample guidelines for information on accurate quantification of human urinary creatinine.

For research use only. Not for diagnostic and clinical purposes.

2. Introduction

Creatine synthesized in kidney, liver, and pancreas is transported in blood to muscle and brain where it is phosphorylated to phosphocreatine. Some free creatine in muscle is converted to creatinine. The amount of creatinine produced is proportional to the individuals muscle mass. In the absence of renal disease, the excretion rate of creatinine in an individual is relatively constant. Thus, urinary creatinine levels may be used as an index of standardization for other tests. Measurement of creatinine clearance is also useful in detecting renal disease and estimating the extent of impairment of renal function (1).

Biosensis' Creatinine (Urinary) Colorimetric Assay can be used to measure creatinine levels in urine. The assay relies on the Jaffe' reaction, wherein a yellow/orange color forms when the metabolite is treated with alkaline picrate (2). The color derived from creatinine is then destroyed at acidic pH. The difference in color intensity measured at 500 nm before and after acidification is proportional to the creatinine concentration (1,3-4). The sample creatinine concentration is determined using a creatinine standard curve.

3. Precautions

Please read these instructions carefully before beginning this assay.

In general, this material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet available on our website.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them may be harmful. The sodium hydroxide and acid solutions are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

The color solution is harmful if swallowed and irritating to eyes, respiratory system, and skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. The color solution is explosive when dry.

4. Materials Provided and Storage Conditions

Reagent	Quantity		
	1 Plate Kit	5 Plate Kit	10 Plate Kit
96 well solid microplate	96 wells	480 wells	960 wells
Creatinine Standard	1 x 3 mL	1 x 15 mL	2 x 15 mL
Creatinine Color Reagent	1 x 12 mL	1 x 60 mL	2 x 60 mL
Creatinine Sodium Hydroxide	1 x 5 mL	1 x 25 mL	2 x 25 mL
Creatinine Acid Solution	1 x 1 mL	1 x 5 mL	2 x 5 mL
Creatinine Sodium Borate	1 x 2.5 mL	1 x 12.5 mL	2 x 12.5 mL
Creatinine Surfactant	1 x 7.5 mL	1 x 37.5 mL	2 x 37.5 mL
Plate sealer	1 cover	5 covers	10 covers

The Creatinine Assay Kit can be stored at room temperature (18-26°C). This kit will perform as specified if stored properly and used before the expiration date indicated on the outside of the box.

5. Equipment Required but Not Supplied

- A plate reader capable of measuring absorbance between 490-500 nm
- Adjustable pipettes, and a repeating pipettor for best results
- A source of pure water; glass distilled water or HPLC-grade water is acceptable

6. Before You Start….

- Read the entire protocol to familiarize yourself with the assay procedure
- If reagents are accidentally stored cold, all reagents must be equilibrated to room temperature before beginning the assay and shaken to dissolve any potential precipitate

7. Urine Sample Preparation

Typically, human urine has creatinine levels in the range of 25 - 400 mg/dL (0.25 - 4 mg/mL, one time collection) or 500-2,000 mg/24 hours.

The preparation of urine samples for analysis has been adapted from the Human Kidney and Urine Proteome Project (HKUPP) workshop (Yamamoto 2010).

- Collect mid-stream urine, preferably of the 2nd morning urine
- Centrifuge for 10 min at 2-8°C at 1,000 x g within 30 minutes of collection
- Analyze immediately or freeze sample aliquots at -20°C to -80°C within 4 hours of collection
- Thaw frozen urine samples at 37°C in a water bath
- Vortex well
- An additional centrifugation step (5 min at 2-8°C at 1,000 x g) may be required if precipitate is observed

If a 24 hour urine sample is desired, collect the total volume of urine over a 24 hour period. Store the pooled urine at 2-8°C until all the collections are taken. If not assaying after all the collections are taken, freeze 5 mL of the pooled 24 hour collection at -80°C. The sample will be stable for at least one month.

Urine should be diluted 1:10 or 1:20 with HPLC-grade water before assaying.

NOTE: The Creatinine (Urinary) Colorimetric Assay is not recommended for plasma or serum samples. Precipitation may occur in the wells upon the addition of the acid solution.

8. Reagent Preparation

Creatinine Standard

The Creatinine Standard contains 20 mg/dl of creatinine in water. It is ready to use to prepare the standard curve. Sufficient Creatinine Standard is provided to prepare two standard curves using the 3 mL size or ten standard curves using the 15 mL size.

Creatinine Color Reagent

The color reagent contains 1.2% picric acid. The picric acid may contain crystals. This is normal and will disappear upon making the Alkaline Picrate Solution (see below).

Creatinine Sodium Hydroxide

The vial contains 1 M sodium hydroxide (NaOH). It is ready to use as supplied.

Creatinine Acid Solution

The acid solution contains a mixture of sulfuric and acetic acid. It is ready to use as supplied.

Creatinine Sodium Borate

The vial contains a solution of sodium borate. It is ready to use as supplied.

Creatinine Surfactant

The vial contains a solution of surfactant. It is ready to used as supplied.

Alkaline Picrate Solution

The volume of Alkaline Picrate Solution needed is dependent on the number of wells being assayed. Calculate 150 µL for each well, i.e., to prepare sufficient reagent for one 96-well plate, mix together:

- 2 mL of Creatinine Sodium Borate
- 6 mL of Creatinine Surfactant
- 10 mL of Creatinine Color Reagent
- 3.6 mL of Creatinine NaOH

The Alkaline Picrate Solution is stable for at least one week stored in the dark at room temperature.

9. Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of Creatinine Standards and samples to be measured in duplicate is given below in Figure 1, below. We suggest you record the contents of each well on the template sheet provided (see Appendix A).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H: Standards; S1-S40: Sample wells

Figure 1: Sample plate format.

10. Standard Preparation

For the determination of creatinine in urine, prepare the Creatinine Standards according to Table 1, below. Take eight clean glass test tubes and label them A-H. Add the amount of Creatinine Standard and HPLC-grade water to each tube as described in Table 1, below.

Table 1: Concentration of standards.

Tube	Creatinine Standard (µL)	HPLC-grade water (µL)	Final creatinine concentration (mg/dL)
A	0	500	**0**
B	50	450	**2**
C	100	400	**4**
D	150	350	**6**
E	200	300	**8**
F	250	250	**10**
G	300	200	**12**
H	375	125	**15**

11. Assay Procedure

General information and technical hints:

- The final volume of the assay is 170 µl in all wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.
- If the concentration of creatinine in the sample is not known or if it is expected to be beyond the range of the standard curve, it is prudent to assay the sample at several dilutions.
- It is recommended that the standards and samples be assayed at least in duplicate (triplicate is recommended).

- Monitor the absorbance at 490-500 nm using a plate reader.
- All steps are performed at room temperature (18-26°C).

Procedure:

1. **Creatinine Standard Wells** - Add 15 µL of Creatinine Standard (tubes A-H) per well in the designed wells on the plate (see suggested plate configuration, Figure 1).
2. **Sample Wells** - Add 15 µL of sample to two wells. To obtain reproducible results, creatinine levels from each sample should fall within the absorbance values of the standard curve. When necessary, samples can be diluted with HPLC-grade water to bring the creatinine concentration to this level.
3. Initiate the reactions by adding 150 µL of Alkaline Picrate Solution to all the wells being used.
4. Cover the plate with the plate cover and incubate on a shaker for 10 minutes at room temperature.
5. Remove the plate cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the **Initial** absorbance reading (I_{abs}).
6. Add 5 µL of acid solution to all of the wells being used.
7. Cover the plate with the plate cover and incubate on a shaker for 20 minutes at room temperature.
8. Remove the cover and read the absorbance at 490-500 nm using a plate reader. This absorbance is the **Final** absorbance reading (F_{abs}).

12. Calculation of Results

1. Calculate the average **Initial** absorbance (I_{abs}) of each standard and sample.
2. Calculate the average **Final** absorbance (F_{abs}) of each standard and sample.
3. Subtract the average Final absorbance from the average Initial absorbance. This is your **Corrected** Absorbance.
4. Subtract the average **Corrected** absorbance of standard A from itself and all other standards and samples. This is the **Adjusted** absorbance.
5. Plot the **Adjusted** absorbance of the standards (from step 4 above) as a function of the final concentration of creatinine from Table 1. See Figure 2 for a typical standard curve.
6. Calculate the creatinine concentration of the samples using the equation obtained from the linear regression of the standard curve substituting **Adjusted** absorbance values for each sample.

Creatinine (mg/dL) = $\frac{[Sample\ absorbance - (y-intercept)]}{Slope}$ x DF

DF = sample dilution factor

NOTE: To convert the results from mg/dL to µmol/L, multiply the creatinine concentration (mg/dL) by 88.4.

13. Typical Data

Standard Curve

The standard curve presented below (Figure 2) is an example of the data typically provided with this kit. However, your results will not be identical to these. A standard curve has to be generated for each creatinine assay.

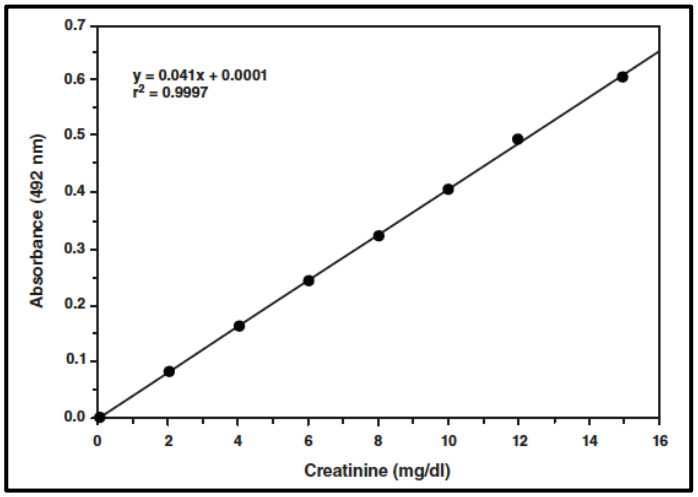


Figure 2: Creatinine standard curve.

Urine samples from 5 healthy individuals were assayed for creatinine and compared to results obtained from a competitor kit (Table 2). Creatinine values closely agreed and fell within the expected range of creatinine in human urine (25 - 400 mg/dL, one time collection).

Table 2: Creatinine concentration in urine.

Sample	mg/dL Biosensis	mg/dL Competitor	% Difference
Urine 1	128	124	3
Urine 2	98	89	9
Urine 3	76	71	7
Urine 4	58	54	7
Urine 5	205	185	10

Precision

Intra-assay coefficient of variation: 2.7% (n = 84).
Inter-assay coefficient of variation: 3.0% (n = 5).

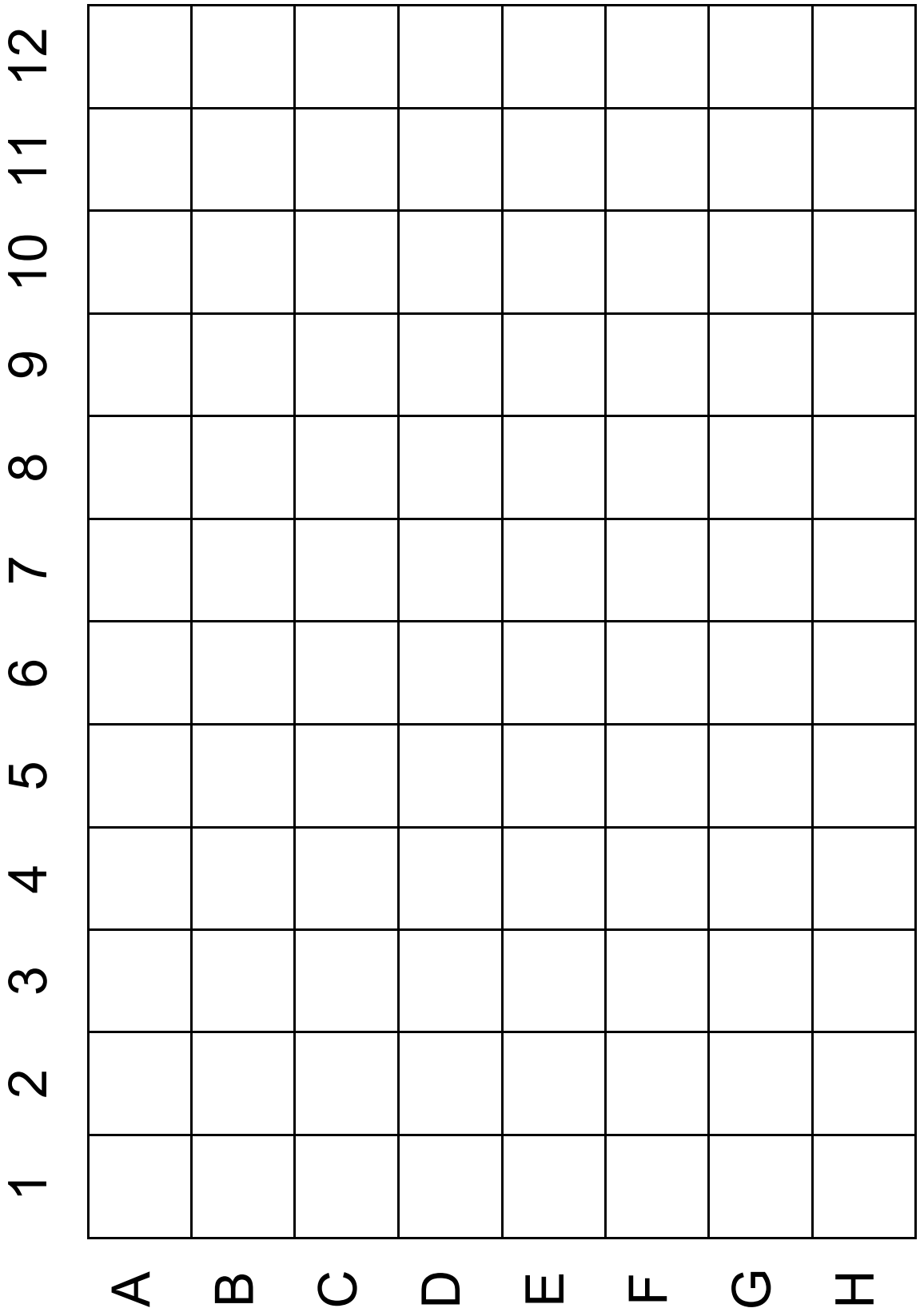
Assay Range

Under the standardized conditions of the assay described in this kit protocol, the dynamic range of the kit is 0-15 mg/dL of creatinine.

14. Informational References

1. Bowers, L.D. and Wong, E.T. *Clin. Chem.* 26(5), 555-561 (1980).
2. Slot, C. *Scand. J. Clin. Lab. Invest.* 17, 381-387 (1965).
3. Heinegård, D. and Tiderström, G. *Clinica. Chimica. Acta.* 43, 305-310 (1973).
4. Cook, J.G.H. *Ann. Clin. Biochem.* 12, 219-232 (1975).

Appendix A: 96 Well Microplate Template



Appendix B: Troubleshooting Guide

This Creatinine Assay Kit has been developed to deliver reproducible results when following the provided assay protocol. Please refer to the troubleshooting guide below when unexpected difficulties are encountered. If you require further assistance, talk to a scientist at Biosensis (biospeak@biosensis.com).

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/ triplicates	A.Poor pipetting/ technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No creatinine was detected in the sample wells	Sample was too dilute	Re-assay the sample using less of a dilution
Sample absorbance values are above highest point in standard curve	Creatinine concentration was too high in the sample	Dilute samples with HPLC-grade water and re-assay.
The creatinine standard curve did not work	Either the creatinine standards were not diluted properly or the creatinine standard has deteriorated	Set up the standards according to table 1 and re-assay