QUIDEL

MicroVue™ Bone Creatinine EIA

An enzyme immunoassay for the quantitation of Creatinine in urine

For **Research Use Only**. Not for use in diagnostic procedures.

SUMMARY

Reagents and Samples Preparation

- ☐ Prepare Color Solution *(add 1 mL Stop Solution to each required vial)*
- ☐ Prepare urine samples according to PI instructions and dilute 1:40 with deionized or distilled water *(e.g. 20 µL sample + 780 µL water)*
- ☐ Dilute Standards and Controls 1:40 with deionized or distilled water *(e.g. 20 µL sample + 780 µL water)*

Assay Procedure

Pipette **50 µL** of each diluted Standard, Control, or urine into assay wells *(Use deionized water as the "0" standard)*

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Carefully pipette **150 µL** of Working Color Solution into assay wells

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Incubate **30 ± 2.5 minutes** at 18°C to 28°C

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Read the Optical Density at **490 nm**
Analyze the assay results using a linear curve fit
(y = mx + b)

SUMMARY AND EXPLANATION

Creatinine is a molecule with a molecular weight of 113.1 g/mol. Creatinine is found in muscle tissues and is excreted into the circulation at a constant rate. It is removed from the plasma by glomerular filtration in the kidney and excreted into the urine.

PRINCIPLE OF THE PROCEDURE

The MicroVue Creatinine assay is a quantitative, colorimetric assay based on a modified Jaffe method where alkaline picrate forms a colored solution in the presence of creatinine.

REAGENTS AND MATERIALS PROVIDED

96 Assays for the Creatinine assay
MicroVue Creatinine assay contains the following:

A **B** **C**	**Creatinine Standard** **(A = 5 mmol/L, B = 20 mmol/L, C = 40 mmol/L)** Liquid Creatinine in phosphoric acid	**Parts 4225-4227**	**0.3 mL each**
L	**Low Control** Liquid Creatinine in phosphoric acid	**Part 4228**	**0.3 mL each**
H	**High Control** Liquid Creatinine in phosphoric acid	**Part 4229**	**0.3 mL each**
❶	**Color Reagent** Picric acid (0.14%) in sodium borate and SDS	**Part 4224**	**7 mL, 3 each**
❷	**Stop Solution** 1N NaOH	**Part 4960**	**15 mL**
	Microassay Plate Eight-well non-coated strips in a frame	**Part 0673**	**12 each**

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60 minute range)
- Tubes for dilution of samples, standards, and controls
- Calculator or other computational method to validate the assay
- Clean, unused microassay plates and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle or other immunoassay washing system
- Adjustable multichannel pipette (8 or 12 channels) or repeating micro-pipettes (optional)
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Micropipettes and pipette tips
- Plate reader capable of optical density readings between 0.0 and 2.0
- Deionized or distilled water

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.

- Store assay reagents as indicated.
- Do not use Strips if pouch is punctured.
- Test each sample in duplicate.
- 1N NaOH may be harmful upon ingestion and can cause irritation to the skin or eyes. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
- The MicroVue Creatinine Color Reagent (picric acid) may be harmful upon ingestion. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician. Do not let picric acid dry as the crystals are explosive. If this solution is spilled, flush with water and dispose of the material in an appropriate manner.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
- For accurate measurement of samples, the addition of samples and standards must be precise. Pipette carefully using only calibrated equipment.
- Perform a standard curve with each assay.
- Creatinine values from nonhuman species have not been evaluated with the MicroVue Creatinine Kit.
- This assay is validated for urine samples only.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

REAGENT PREPARATION

Bring all reagents and materials to 18°C to 28°C before use.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*).

Refer to Table 1 for the amounts of reagents and materials required per number of wells.

Table 1
Determine amount of Color Reagent required for the number of strips to be used.

# of Strips	**2**	**4**	**6**	**8**	**10**	**12**
# of Samples (tested in duplicate)	**2**	**10**	**18**	**26**	**34**	**42**
Color Reagent (bottles)	1	1	1	2*	2*	2*

*When more than one bottle or vial is to be used, combine the contents and mix prior to use.

Working Color Solution

Prepare Working Color Solution by adding 1 mL of Stop Solution to each required bottle of Color Reagent (see Table in *ASSAY PROCEDURE* section). Color Reagent should be at room temperature prior to use. If a precipitate develops, warm the bottle at low temperature (37°C) to dissolve the precipitate, but equilibrate to room temperature before use.

STORAGE

Store the unopened kit at 2°C to 8°C. All reagents must be brought to 18°C to 28°C before use.

SPECIMEN COLLECTION AND STORAGE

Handle and dispose of all specimens using Universal Precautions.

Keep the urine samples at 2°C to 8°C if testing within 2 to 3 days of collection. Store at –20°C for longer storage. Do not subject samples to more than four (4) freeze/thaw cycles.

ASSAY PROCEDURE

Read entire product Insert before beginning the assay.
See REAGENT PREPARATION and WARNINGS AND PRECAUTIONS before proceeding.

Sample Preparation

1. Mix urine samples and allow to settle for 15 to 20 minutes, or centrifuge at low speed (200 xg) for at least 2 minutes. This prevents debris from interfering during pipetting.
2. Dilute Standards, Controls, and urine samples 1:40 with deionized or distilled water (e.g. 20 µL sample + 780 µL water). Use deionized or distilled water as the zero "0" standard.
3. Place desired number of 1 x 8 strips in the Stripwell Frame.
4. Prepare Working Color Solution by adding 1 mL of Stop Solution to each required bottle of Color Reagent (see Table above). Color Reagent should be at room temperature prior to use. If a precipitate develops, warm the bottle at low temperature (37°C) to dissolve the precipitate, but equilibrate to 18°C to 28°C before use

Incubation

5. Add 50 µL of diluted Standard (0 [water], A, B, C), Control or urine sample to each well of the 1 x 8 strips in the frame.
6. Carefully add 150 µL of the Working Color Solution to each well.
7. Incubate 30 ± 2.5 minutes at 18°C to 28°C.

Read

8. Read the optical density at 490 nm. Assure that no large bubbles are present in wells and that the bottoms of the strips are clean. Read strips within **10 minutes** of completion of the incubation step.
9. Use quantitation software with a linear regression equation to analyze the MicroVue Creatinine assay results.
10. Determine concentration of samples and Controls from the standard curve.
11. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

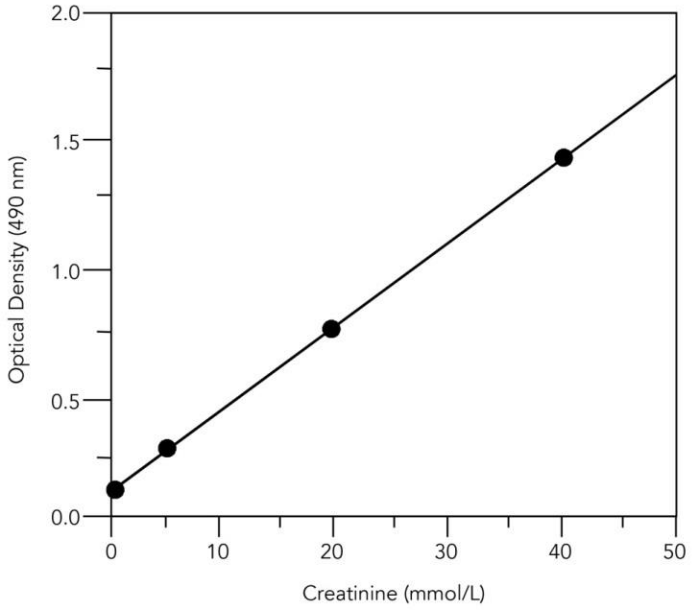
QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation.
Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

INTERPRETATION OF RESULTS

Representative Standard Curve

Standard Creatinine levels: 0, 5, 20, 40 mmol/L



PERFORMANCE OF THE TEST

Precision

Within-run and between-run precision were determined by assaying six urine samples in ten different runs. Typical results are provided below.

Creatinine (nmol/L)	Within-run[1] C.V. (%)	Between-run[2] C.V. (%)
4.8	1.6	6.9
8.3	2.1	4.6
13.1	1.7	1.6
17.1	1.4	5.4
22.8	2.0	2.0
28.3	1.1	5.4

[1]n = 40 replicates [2]n = 10 runs

Recovery – Linearity

Linearity was determined by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

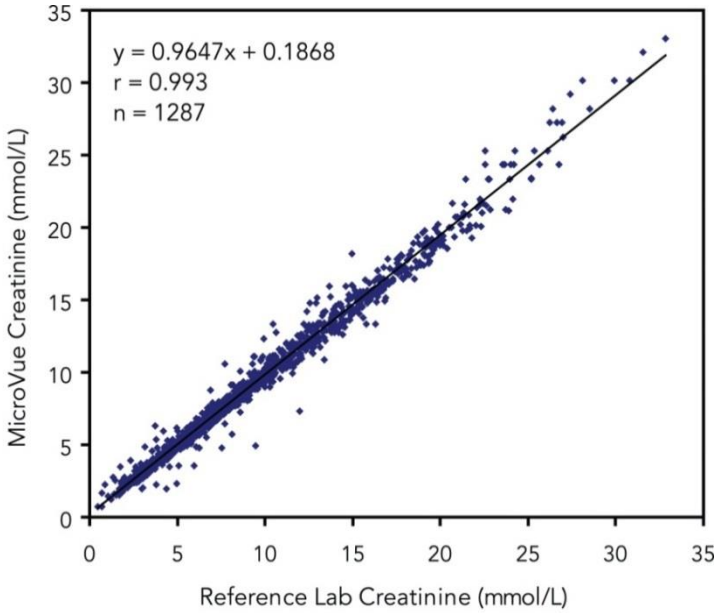
Sample	Dilution Factor	Observed (mmol/L)	Expected (mmol/L)	Recovery (%)
1	neat	36.2	–	–
	1:2	18.4	18.1	102
	1:4	9.1	9.1	100
	1:8	4.5	4.5	100
2	neat	32.2	–	–
	1:2	16.3	16.1	101
	1:4	8.3	8.1	102
	1:8	4.1	4.0	103
3	neat	22.3	–	–
	1:2	11.2	11.2	100
	1:4	5.4	5.6	96
	1:8	2.5	2.8	89

Recovery – Spike Recovery

Sample	Endogenous (mmol/L)	Added (mmol/L)	Observed (mmol/L)	Recovery (%)
1	3.9	8.4	12.8	106
2	11.0	8.4	20.4	112
3	14.7	8.4	23.8	108

Accuracy

Accuracy was determined by comparing creatinine values with those assayed by autoanalyzer at a reference laboratory.



ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

REFERENCES

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REF 8009 – MicroVue Creatinine EIA Kit

RUO

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PI8009000EN00 (12/16)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
 Biological risks	RUO For Research use only
 Contains sufficient for 96 determinations	CONT Contents/Contains
CONTROL Control	