Technical Bulletin

Creatinine Assay Kit

Catalogue number MAK475

Product Description

Creatinine is synthesized in the body from creatine, which is produced from creatine phosphate during muscle contractions. In the blood, creatinine is removed by filtration through the glomeruli of the kidney and is secreted into urine. In healthy individuals, creatinine secretion is independent of diet and is fairly constant. The creatinine clearance test has become one of the most sensitive tests for measuring glomerular filtration rate.

The Creatinine Assay Kit uses a reaction sequence to convert a dye into a colored and fluorescent form. The absorbance at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is directly proportional to the creatinine concentration in the sample. The reaction specifically excludes both endogenous creatine and ammonia.

The linear detection range of creatinine is 4.8 – 500 µM (0.054 - 5.7 mg/dL) for the colorimetric assay and 0.25 – 100 µM (0.0028 - 1.14 mg/dL) for the fluorometric assay. The kit is suitable for creatinine determination in urine, serum, plasma, and other biological preparations.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 20 mL
  Catalogue Number MAK475A
- Enzyme A — 1 Vial
  Catalogue Number MAK475B
- Enzyme B — 1 Vial
  Catalogue Number MAK475C
- Dye Reagent — 120 µL
  Catalogue Number MAK475D
- Standard (2 mM) — 1 mL
  Catalogue Number MAK475E

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor, pipette tips, etc.)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Corning® Spin-X® UF concentrators (Catalogue Number CLS431478)
- Microcentrifuge capable of RCF ≥ 14,000 × g

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate all reagents to room temperature prior to use.

Enzyme A: Reconstitute the vial with 120 µL of Assay Buffer. Pipette up and down until completely dissolved. The reconstituted Enzyme Mix is stable for at least 1 month when stored at -20 °C.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Urine

Urine samples should be diluted at least 100-fold with purified water prior to assay.

Serum and Plasma

Serum and plasma samples should be deproteinated by centrifugation for 15 minutes at 14,000 × g at room temperature through a 10 kDa spin filter. The filtrate can be assayed directly.

Colorimetric Standard Curve Reaction

1. Prepare a 500 µM Creatinine Standard by mixing 50 µL of Standard (2 mM) and 150 µL of purified water.
2. Prepare Creatinine standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Colorimetric Creatinine Standards

Well No.	500 µM Standard	Purified Water	Creatinine (µM)
1	100 µL	–	500
2	60 µL	40 µL	300
3	30 µL	70 µL	150
4	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96 well plate.

Fluorometric Standard Curve Preparation

1. Dilute the Standards prepared in Colorimetric Procedure (see Table 1) 1:5 in purified water according to Table 2.

Table 2.
Preparation of Fluorometric Creatinine Standards

Well	Colorimetric Standard	Purified Water	Creatinine (µM)
1	20 µL of 500 µM Std	80 µL	100
2	20 µL of 300 µM Std	80 µL	60
3	20 µL of 150 µM Std	80 µL	30
4	-	100 µL	0

Mix well and transfer 20 µL of each Standard into separate wells of a black 96 well plate.

Working Reagent

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 85 µL of Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	82 µL
Enzyme A	1 µL
Enzyme B	1 µL
Dye Reagent	1 µL

2. Transfer 80 µL of Working Reagent into each Standard and Sample well. Tap plate to mix.

Measurement

Incubate at room temperature (protect plate from light for fluorometric assay) for 60 minutes. Measure the absorbance (OD) at 570 nm or fluorescence (RFU) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Subtract the blank (Standard #4) absorbance (OD) or fluorescence (RFU) value from the remaining Standard values.
2. Plot the adjusted values (ΔOD or ΔRFU) against standard concentrations and determine the slope of the standard curve (μM^{-1}).
3. Calculate the creatinine concentration of Sample using the below equation:

$$\text{Creatinine } (\mu M) = \left(\frac{R_{Sample}-R_{Blank}}{\text{Slope}(\mu M^{-1})} \times DF\right)$$

Where:

R_{Sample} = Absorbance (OD) or fluorescence (RFU) value of Sample

R_{Blank} = Absorbance (OD) or fluorescence (RFU) value of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Conversions: 1 µM creatinine equals 0.0113 mg/dL.

Figure 1.

Typical Creatinine Standard Curve (Colorimetric Assay)

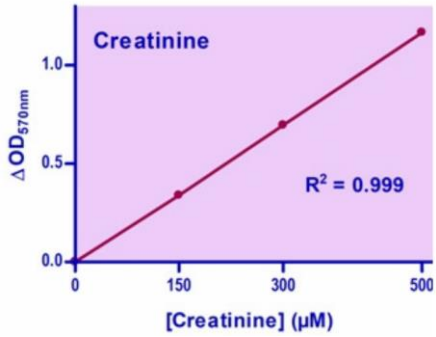
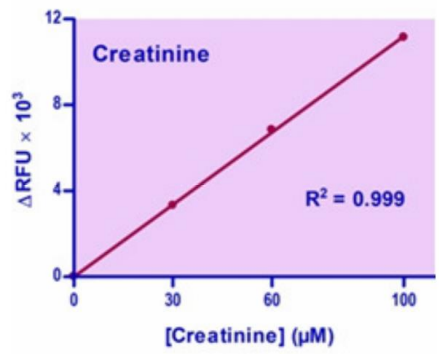


Figure 2.

Typical Creatinine Standard Curve (Fluorometric Assay)



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak475pis Rev 12/23