

## Product Information

### Creatinine Assay Kit

Catalog Number **MAK080**  
Storage Temperature  $-20^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

Creatinine is generated from creatine by nonenzymatic dehydration. Creatinine is produced at a constant rate and is excreted from the body through kidney glomerular filtration. Decreased kidney function can effect the rate at which creatinine is filtered by the kidneys and can be used as a measure of kidney function. Decreased kidney function can result in increased serum creatinine levels due to the inability to clear creatinine through urine excretion. Creatinine levels can be affected by changes in muscle mass, pregnancy, or the use of angiotensin inhibitors or angiotensin receptor antagonists.

In this assay, creatinine concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric ( $\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587$  nm) product, proportional to the creatinine present. This kit is suitable for use with cell and tissue culture supernatants as well as serum, plasma, urine, and other biological fluids.

### Components

The kit is sufficient for 100 assays in 96 well plates.

Creatinine Assay Buffer Catalog Number MAK080A	25 mL
Creatinine Probe, in DMSO Catalog Number MAK080B	0.2 mL
Creatinase Catalog Number MAK080C	1 mL
Creatininase Catalog Number MAK080D	1 mL
Creatinine Enzyme Mix Catalog Number MAK080E	1 mL
Creatinine Standard, 10 $\mu\text{mole}$ Catalog Number MAK080F	1 mL

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Creatinine Assay Buffer – Allow buffer to come to room temperature before use.

Creatinine Probe – Thaw completely at room temperature to melt frozen DMSO prior to use. Aliquot and store protected from light and moisture at  $-20^{\circ}\text{C}$ . Upon complete thawing, the Creatinine Probe is ready-to-use in the colorimetric assay. **Note:** It is advised to centrifuge the vial to consolidate the material after thawing.

For the fluorescence assay, dilute an aliquot of the Creatinine Probe 5 to 10-fold with Creatinine Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Creatininase, Creatinase, and Creatinine Enzyme Mix – Reconstitute each with 220  $\mu\text{L}$  of Creatinine Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at  $-20^{\circ}\text{C}$ . Use within 2 months of reconstitution and keep cold while in use.

Creatinine Standard – Reconstitute in 100  $\mu\text{L}$  of water to generate a 100 mM (100 nmole/ $\mu\text{L}$ ) Creatinine Standard solution. Mix well by pipetting, then aliquot and store at  $-20\text{ }^{\circ}\text{C}$ . Use within 2 months of reconstitution and keep cold while in use.

### Storage/Stability

The kit is shipped on wet ice. Storage at  $-20\text{ }^{\circ}\text{C}$ , protected from light, is recommended.

### Procedure

All samples and standards should be run in duplicate.

#### Creatinine Standards for Colorimetric Detection

Dilute 10  $\mu\text{L}$  of the 100 mM (100 nmole/ $\mu\text{L}$ ) Creatinine Standard Solution with 990  $\mu\text{L}$  of Creatinine Assay Buffer to prepare a 1 mM (1 nmole/ $\mu\text{L}$ ) standard solution. Add 0, 2, 4, 6, 8, 10  $\mu\text{L}$  of the 1 mM Creatinine standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Creatinine Assay Buffer to each well to bring the volume to 50  $\mu\text{L}$ .

#### Creatinine Standards for Fluorometric Detection

Dilute 10  $\mu\text{L}$  of the 100 mM (100 nmole/ $\mu\text{L}$ ) Creatinine Standard Solution with 990  $\mu\text{L}$  of Creatinine Assay Buffer to prepare a 1 mM (1 nmole/ $\mu\text{L}$ ) standard solution. Dilute 10  $\mu\text{L}$  of the 1 mM standard solution with 90  $\mu\text{L}$  of water to generate a 0.1 mM (0.1 nmole/ $\mu\text{L}$ ). Add 0, 2, 4, 6, 8, 10  $\mu\text{L}$  of the 0.1 mM Creatinine standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Creatinine Assay Buffer to each well to bring the volume to 50  $\mu\text{L}$ .

#### Sample Preparation

Tissue (10 mg) or cells ( $2 \times 10^6$ ) should be rapidly homogenized in 4 volumes of cold Creatinine Assay buffer. Centrifuge at  $13,000 \times g$  for 10 minutes at  $4\text{ }^{\circ}\text{C}$  to remove insoluble material. High concentrations of proteins may interfere with the assay and should be removed with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Serum samples may be deproteinized with a 10 kDa MWCO spin filter. The soluble fraction may be assayed directly.

Bring samples to a final volume of 50  $\mu\text{L}$  with Creatinine Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Sarcosine and creatine present in the sample can generate background. To control for sarcosine and creatine background, include a blank sample for each sample by omitting the Creatininase in the Reaction Mix.

Compounds in the sample may interfere with the assay. To test for interference, samples can be spiked with a known amount of creatinine (1–10 nmole) and compared to unspiked samples to ensure readings are accurate.

#### Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50  $\mu\text{L}$  of the appropriate Reaction Mix is required for each reaction (well).

**Table 1.**  
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
Creatinine Assay Buffer	44 $\mu\text{L}$	42 $\mu\text{L}$
Creatinase	2 $\mu\text{L}$	2 $\mu\text{L}$
Creatininase	–	2 $\mu\text{L}$
Creatinine Enzyme Mix	2 $\mu\text{L}$	2 $\mu\text{L}$
Creatinine Probe	2 $\mu\text{L}$	2 $\mu\text{L}$

2. Add 50  $\mu\text{L}$  of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at  $37\text{ }^{\circ}\text{C}$ . Protect the plate from light during the incubation.
3. For colorimetric assays, measure the absorbance at 570 nm ( $A_{570}$ ). For fluorometric assays, measure fluorescence intensity ( $\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587\text{ nm}$ ).

## Results

### Calculations

The background for the assays is the value obtained for the 0 (blank) Creatinine Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Creatinine standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of creatinine present in the sample may be determined from the standard curve

### Concentration of Creatinine

$$S_a/S_v = C$$

$S_a$  = Amount of Creatinine in unknown sample (nmole)  
from standard curve

$S_v$  = Sample volume ( $\mu\text{L}$ ) added into the wells

$C$  = Concentration of Creatinine in sample

Creatinine molecular weight: 113.12 g/mole

### Sample Calculation

Amount of Creatinine ( $S_a$ ) = 5.84 nmole  
(from standard curve)

Sample volume ( $S_v$ ) = 50  $\mu\text{L}$

Concentration of Creatinine in sample

$$5.84 \text{ nmole}/50 \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 113.12 \text{ ng/nmole} = 13.21 \text{ ng}/\mu\text{L}$$

**Troubleshooting Guide**

<b>Problem</b>	<b>Possible Cause</b>	<b>Suggested Solution</b>
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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