

**Enzymatic Assay of CREATININE DEIMINASE  
(EC 3.5.4.21)**

**PRINCIPLE:**

Creatinine + H<sub>2</sub>O  $\xrightarrow{\text{Creatinine Deiminase}}$  N-Methylhydantoin + NH<sub>3</sub>

NH<sub>4</sub><sup>+</sup> + α-Ketoglutarate + β-NADPH  $\xrightarrow{\text{GDH}}$  Glutamate + H<sub>2</sub>O + β-NADP

Abbreviations used:

β-NADPH = β-Nicotinamide Adenine Dinucleotide Phosphate,  
Reduced Form

GDH = L-Glutamic Dehydrogenase (NADP)

β-NADP = β-Nicotinamide Adenine Dinucleotide Phosphate,  
Oxidized Form

**CONDITIONS:** T = 37°C, pH = 7.5, A<sub>340nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 50 mM Creatinine Solution (Creatinine)  
(Prepare 25 ml in Reagent A using Creatinine, Free Base, Anhydrous, Sigma Prod. No. C-4255.)
- C. 3.0 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form Solution (β-NADPH)  
(Dissolve the contents of one 5 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form, Tetrasodium Salt, Sigma Stock No. 201-205, in the appropriate volume of Reagent A. **PREPARE FRESH.**)
- D. 10.0 mM α-Ketoglutarate Solution (α-KG)  
(Prepare 3 ml in Reagent A using α-Ketoglutaric Acid, Free Acid, Sigma Prod. No. K-1750. **PREPARE FRESH.**)
- E. L-Glutamic Dehydrogenase (NADP) Enzyme Solution (GDH)  
(Immediately before use, prepare a solution containing 1000 units/ml of L-Glutamic Dehydrogenase (NADP), Sigma Prod. No. G-4387, in cold deionized water.)

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**REAGENTS:**

F. Creatinine Deiminase Enzyme Solution (Creat Deiminase)  
(Immediately before use, prepare a solution containing  
0.06 - 0.12 unit/ml of Creatinine Deiminase in cold  
Reagent A.)

**PROCEDURE:**

Pipette (in milliliters) the following reagents into  
suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent B (Creatinine)	2.40	2.40
Reagent C (β-NADPH)	0.30	0.30
Reagent D (α-KG)	0.30	0.30
Reagent E (GDH)	0.05	0.05

Mix by inversion and equilibrate to 37°C. Monitor the  
A<sub>340nm</sub> until constant, using a suitably thermostatted  
spectrophotometer. Then add:

Reagent F (Creat Deiminase)	0.10	-----
Reagent A (Buffer)	-----	0.10

Immediately mix by inversion and record the decrease in  
A<sub>340nm</sub> for approximately 5 minutes. Obtain the r A<sub>340nm</sub>/minute  
using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(r A_{340nm}/\text{min Test} - r A_{340nm}/\text{min Blank})(3.15)(df)}{(6.22)(0.1)}$$

3.15 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADPH  
at 340 nm

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

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**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu$ mole of creatinine to N-methylhydantoin and  $\text{NH}_3$  per minute at pH 7.5 at 37°C in a coupled system with L-glutamic dehydrogenase.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.15 ml reaction mix, the final concentrations are 49 mM potassium phosphate, 38 mM creatinine, 0.29 mM  $\beta$ -nicotinamide adenine dinucleotide phosphate, reduced form, 0.95 mM  $\alpha$ -ketoglutaric acid, 50 units L-glutamic dehydrogenase (NADP), and 0.006 - 0.012 unit creatinine deiminase.

**REFERENCE:**

Esders, T.W. and Lynn, S.Y. (1985) *Journal of Biological Chemistry* **260**, 3915-3922

**NOTES:**

1. This assay is based on the cited reference.
2. L-Glutamic Acid Dehydrogenase (NADP) Unit Definition:  
One unit will reduce 1.0  $\mu$ mole of  $\alpha$ -ketoglutarate to L-glutamate per minute at pH 8.3 at 30°C in the presence of ammonium ions and NADPH.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**