

## SPECIFICATION SHEET

**Product Name: Creatinine Amidohydrolase**
**Catalog # CR01D1 -E311H**

**Product Description:** Creatinine amidohydrolase is a diagnostic grade reagent from a microorganism host. This enzyme is useful for enzymatic determination of creatinine when coupled with creatine amidinohydrolase and sarcosine oxidase in clinical analysis.

**EC 3.5.2.10**

**Storage and Stability:** Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

**Principle:**

Creatine  $\xrightarrow{\text{creatinine amidohydrolase}}$  Creatinine + H<sub>2</sub>O

Creatinine + Picric acid  $\xrightarrow{\text{OH}^-}$  Orange dye

The appearance of creatinine-picrate (orange dye based on Jaffe's reaction) is measured at 520 nm by spectrophotometry.

|                                    |                                                                                              |                                     |
|------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------|
| <b>Unit Definition</b>             | : One unit causes the formation of one micromole of orange dye per minute at pH 7.5 and 37°C |                                     |
| <b>Appearance</b>                  | : White amorphous powder, lyophilized                                                        |                                     |
| <b>Activity</b>                    | : ≥300 U/mg solid                                                                            |                                     |
| <b>Molecular Weight</b>            | : 29.7 kDa                                                                                   |                                     |
| <b>Contaminants</b>                | : Catalase<br>NADH oxidase                                                                   | ≤1.0 %<br>≤5.0 × 10 <sup>-2</sup> % |
| <b>Stabilizers</b>                 | : Sucrose, BSA                                                                               |                                     |
| <b>Isoelectric Point</b>           | : 5.64                                                                                       |                                     |
| <b>Michaelis Constant</b>          | : 5.5 × 10 <sup>-2</sup> M (Creatine), 4.9 × 10 <sup>-2</sup> M (Creatinine)                 |                                     |
| <b>Inhibitors</b>                  | : Ag <sup>+</sup> , Hg <sup>2+</sup>                                                         |                                     |
| <b>Optimum pH</b>                  | : 6.0 ~ 8.0                                                                                  | (Figure 1)                          |
| <b>Optimum Temperature</b>         | : 50°C                                                                                       | (Figure 2)                          |
| <b>pH Stability</b>                | : pH 7.5~10.5 (37°C, 17 hr)                                                                  | (Figure 3)                          |
| <b>Thermal Stability</b>           | : Below 65°C (pH 7.5, 30 min)                                                                | (Figure 4)                          |
| <b>Effect of Various Chemicals</b> | : (Table 1)                                                                                  |                                     |

**Table 1. Effect of Various Chemicals on Creatinine Amidohydrolase**

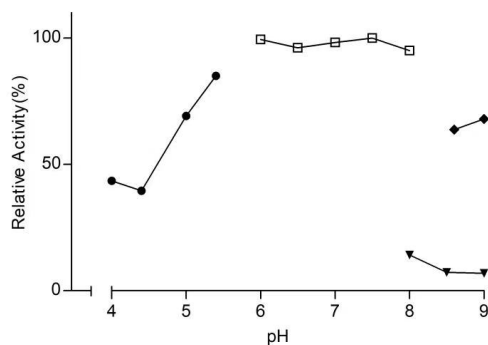
The enzyme dissolved in 50 mM Tris-HCl buffer, pH 8.0 (ca.20 U/mL) was incubated with each chemical at 37°C for 2 hr.

| Chemical          | Conc. (mM) | Residual activity (%) | Chemical         | Conc. (mM)   | Residual activity (%) |
|-------------------|------------|-----------------------|------------------|--------------|-----------------------|
| None              | —          | 100                   | BME              | 2            | 96                    |
| CaCl <sub>2</sub> | 2          | 89                    | Hydroxylamine    | 2            | 90                    |
| MgSO <sub>4</sub> | 2          | 97                    | EDTA             | 5            | 87                    |
| ZnSO <sub>4</sub> | 2          | 182                   | NaF              | 20           | 88                    |
| NiCl <sub>2</sub> | 2          | 87                    | NaN <sub>3</sub> | 20           | 90                    |
| CoCl <sub>2</sub> | 2          | 225                   | Proclin-300      | 0.045% (v/v) | 91                    |
| MnCl <sub>2</sub> | 2          | 111                   | SDS              | 0.05% (w/v)  | 95                    |
| FeCl <sub>3</sub> | 2          | 90                    | Na-Cholate       | 0.1% (w/v)   | 109                   |

**Table 1 (Continued)**

| Chemical          | Conc. (mM) | Residual activity (%) | Chemical     | Conc. (mM) | Residual activity (%) |
|-------------------|------------|-----------------------|--------------|------------|-----------------------|
| CuSO <sub>4</sub> | 2          | 91                    | Tween-20     | 0.1% (v/v) | 104                   |
| AgNO <sub>3</sub> | 2          | 3                     | Triton X-100 | 0.1% (v/v) | 107                   |
| HgSO <sub>4</sub> | 2          | 2                     | Span-20      | 0.1% (v/v) | 100                   |
| NEM               | 2          | 92                    | Brij-35      | 0.1% (w/v) | 106                   |
| IAA               | 2          | 91                    |              |            |                       |

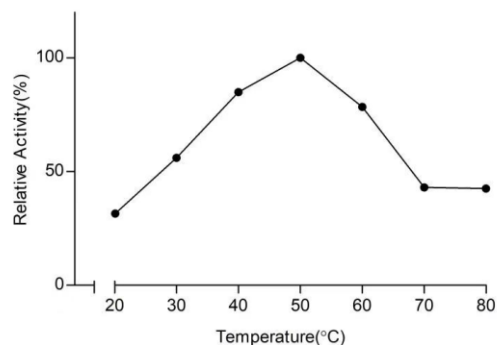
**Fig.1. pH-Activity**



37°C in the following solution:

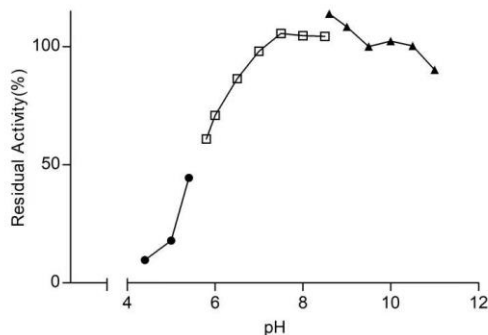
- 50mM Acetate buffer
- 50mM K-phosphate buffer
- ▼ 50mM Tris-HCl buffer
- ◆ 50mM Carbonate buffer

**Fig.2. Temperature Activity**



in 50mM K-phosphate buffer, pH7.4

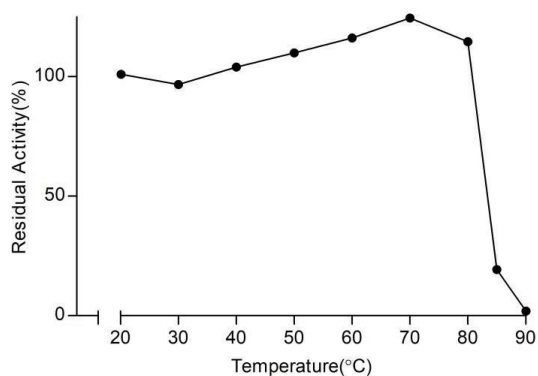
**Fig.3. pH Stability**



25°C 17hr-treatment with the following solution:

- 50mM Acetate buffer
- 50mM K-phosphate buffer
- ▲ 50mM Carbonate buffer

**Fig.4. Thermal Stability**



30min- treatment with 50mM K-phosphate buffer, pH7.4