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YUANYE QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of SULFATASE¹ (EC 3.1.6.1)

PRINCIPLE:

p-Nitrocatechol Sulfate + H₂O $\xrightarrow{\text{Sulfatase}}$ p-Nitrocatechol + Sulfate

CONDITIONS: T = 37°C, pH = 5.0, A_{515nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 200 mM Sodium Acetate Buffer, pH 5.0 at 37°C
(Prepare 50 ml in deionized water using Sodium Acetate, Trihydrate. Adjust to pH 5.0 at 37°C with 5 M HCl.)
- B. 6.25 mM p-Nitrocatechol Sulfate Solution (PNCS)
(Prepare 10 ml in deionized water using p-Nitrocatechol Sulfate, Dipotassium Salt.)
- C. 1 M Sodium Hydroxide (NaOH)
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous.)
- D. 0.2% (w/v) Sodium Chloride Solution
(Prepare 50 ml in deionized water using Sodium Chloride.)
- E. Sulfatase Enzyme Solution
(Immediately before use, prepare a solution containing 2.5 - 5.0 units/ml of Sulfatase in cold Reagent D.)

PROCEDURE:

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

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Reagent A (Buffer) | 0.50 | 0.50 |
| Reagent B (PNCS) | 0.40 | 0.40 |



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PROCEDURE: (continued)

Mix by swirling and equilibrate to 37°C. Then add:

| | | |
|-----------------------------|------|-------|
| Reagent E (Enzyme Solution) | 0.10 | ----- |
|-----------------------------|------|-------|

Mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

| | | |
|-----------------------------|-------|------|
| Reagent C (NaOH) | 5.00 | 5.00 |
| Reagent E (Enzyme Solution) | ----- | 0.10 |

Immediately mix by swirling. Transfer the solutions to suitable cuvettes and record the $A_{515\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{515\text{nm}} \text{ Test} - A_{515\text{nm}} \text{ Blank})(2)(\text{df})(6)}{(12.6)(0.1)}$$

2 = Time factor correction (Unit Definition Time = 1 hour)

df = Dilution factor

6 = Total volume (in milliliters) of the assay

12.6 = Millimolar extinction coefficient of 4-nitrocatechol at 515 nm.

0.1 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of p-nitrocatechol sulfate per hour at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 100 mM sodium acetate, 2.5 mM p-nitrocatechol, 0.02% (w/v) sodium chloride, and 0.25 - 0.50 unit of sulfatase.