

**Enzymatic Assay of SULFATASE
(EC 3.1.6.1)
from Aerobacter aerogenes**

PRINCIPLE:

p-Nitrophenyl Sulfate + H₂O $\xrightarrow{\text{Sulfatase}}$ p-Nitrophenol + Sulfate
CONDITIONS: T = 37°C, pH = 7.1, A_{420nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 200 mM Tris Buffer Solution, pH 7.1 at 37°C
(Use Trizma Buffer Solution, Sigma Stock No. 106-74.)
- B. 120 mM p-Nitrophenyl Sulfate Solution
(Prepare 10 ml in deionized water using p-Nitrophenyl Sulfate, Potassium Salt, Sigma Prod. No. N-3877.)
- C. Sulfatase Enzyme Solution
(Immediately before use, prepare a solution containing 0.5 unit/ml of Sulfatase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.80
Reagent B (p-Nitrophenyl Sulfate)	0.20	0.20

Mix by inversion and equilibrate to 37°C. Monitor the A_{420nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution)	0.10	-----
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PROCEDURE: (continued)

Immediately mix by inversion and record the increase in the $A_{420\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{420\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{420\text{nm}}/\text{min Test} - \Delta A_{420\text{nm}}/\text{min Blank})(3.0)(\text{df})}{(9.5)(0.10)}$$

3.0 = Total volume (in milliliters) of assay

df = Dilution factor

9.5 = Millimolar extinction coefficient of p-nitrophenol at

420 nm^{-1}

0.10 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of p-nitrophenyl sulfate per minute at pH 7.1 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 187 mM Tris, 8.0 mM p-nitrophenyl sulfate and 0.05 unit of sulfatase.

REFERENCE:

Fowler, L. R. and Rammler D. H. (1964) *Biochemistry* 3, 230-237

NOTES:

1. The millimolar extinction coefficient has been

determined experimentally by Sigma.

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NOTES: (continued)

2. Sulfatase is reported to be inhibited by phosphate as described in the cited reference.
3. This assay is based on the cited reference.
4. 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.