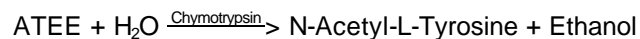


**Suitability Assay of 4-(2-AMINOETHYL)BENZENESULFONYL
FLUORIDE HYDROCHLORIDE as a Serine Protease Inhibitor**

PRINCIPLE:

The reaction shown below is inhibited by AEBSF



Abbreviations used:

AEBSF = 4-(2-Aminoethyl)Benzenesulfonyl Fluoride

ATEE = N-Acetyl-L-Tyrosine Ethyl Ester

CONDITIONS: T = 25°C, pH = 7.0, $A_{237\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- B. 50 mM Potassium Phosphate Buffer, pH 7.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.0 at 25°C with Reagent A.)
- C. 1.0 mM N-Acetyl-L-Tyrosine Ethyl Ester Solution (ATEE)
(Prepare 20 ml in Reagent B using N-Acetyl-L-Tyrosine Ethyl Ester, Sigma Prod. \ No. A-6751.)
- D. 4.2 mM 4-(2-Aminoethyl)Benzenesulfonyl Fluoride Solution (AEBSF)
(Prepare 10 ml in deionized water using 4-(2-Aminoethyl)Benzenesulfonyl Fluoride Solution.)
- E. a-Chymotrypsin Enzyme Solution
(Immediately before use, prepare a solution containing approximately 8 units/ml of a-Chymotrypsin, Sigma Prod. No. C-4129, in cold deionized water.)

**Suitability Assay of 4-(2-AMINOETHYL)BENZENESULFONYL
FLUORIDE HYDROCHLORIDE as a Trypsin Inhibitor**

PROCEDURE:

Step 1:

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

	<u>Test 1</u> <u>Inhibited</u>	<u>Test 2</u> <u>Uninhibited</u>	<u>Blank</u>
Reagent E (Enz Soln)	2.00	2.00	-----
Reagent D (AEBSF)	0.20	-----	-----
Deionized Water	1.80	2.00	-----

Mix by swirling and incubate at 25°C for exactly 1 minute. Then proceed with Step 2.

Step 2:

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

Reagent C (ATEE)	3.00	3.00	3.00
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Equilibrate to 25°C. Then add:

Test 1 (from Step 1)	-----	-----	-----
Test 2 (from Step 1)	0.10	-----	-----
Reference (from Step 1)	-----	0.10	-----
Deionized Water	-----	-----	0.10

Immediately mix by inversion and record the decrease in A_{237nm} for approximately 5 minutes. Obtain the $\Delta A_{237nm}/\text{minute}$ using the maximum linear rate for Test 1, Test 2, and the Reference Solution.

CALCULATIONS:

$$\% \text{ Inhibition} = 1 - \left[\frac{r A_{237nm} / \text{min Test}_1 - r A_{237nm} / \text{min Blank}}{(r A_{237nm} / \text{min Test}_2 (\text{uninhibited}) - r A_{237nm} / \text{min Blank})} \right] \times 100\%$$

SPECIFICATION:

Compare the % inhibition of serine protease activity to that of a control. The values should be similar.

**Suitability Assay of 4-(2-AMINOETHYL)BENZENESULFONYL
FLUORIDE HYDROCHLORIDE as a Trypsin Inhibitor**

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 48 mM potassium phosphate, 0.007-0.07 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 0.97 mM N-acetyl-L-tyrosine ethyl ester, and 0.4 unit a-chymotrypsin.

REFERENCE:

Lawson, W.B., Valenty, V.B., Wos, J.D. and Lobo, A.P. (1982) *Folia Haematologica* **109**, S. 52-60

NOTES:

1. This assay is based on the cited reference.
2. 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.