AEBSF

Catalog Number: EI001
Lot Number: 1384335

Specifications and Use

Product	♦ 4-(2-Aminoethyl-benzensulfonyl fluoride hydrochloride) (AEBSF)
Molecular Mass	♦ 239.7 Da
Purity	♦ > 96% by high performance liquid chromatography
Quantity	♦ 250 mg
Effective Concentration	♦ 0.1 - 1.0 mM
Activity and Applications	♦ Measured by its ability to inhibit trypsin cleavage of a peptide substrate (R&D Systems, Catalog # ES002). ♦ The IC_{50} is < 15 µM, as measured under the described conditions. See Activity Assay Protocol on next page for details. ♦ AEBSF is an irreversible inhibitor of serine proteases. As compared to PMSF (Phenylmethanesulphonyl fluoride), which is another commonly used serine protease inhibitor, AEBSF is water soluble, less toxic and more stable.[1] ♦ AEBSF also inhibits NADH oxidase activation through a non-proteolytic route.[2]
Formulation	♦ Powder was obtained from a solvent containing absolute ethanol and ether.
Reconstitution	♦ It is recommended that small amounts of powder be weighed and dissolved in water to give a stock solution at 100 mM. Aliquot and store at -20° C **in a manual defrost freezer.**
Storage	♦ Powder is stable for up to twelve months from date of receipt at -20° C to -70° C. ♦ Upon reconstitution, the samples can be stored at 2° - 8° C for 1 - 2 months or at -20° C to -70° C **in a manual defrost freezer** for three months. ♦ **Avoid repeated freeze-thaw cycles.**

References:

1. Beynon, R. and J.S. Bond, 2001, *Proteolytic Enzymes: A Practical Approach*, Oxford University Press.
2. Diatchuk, V. *et al*. 1997, J. Biol. Chem. **272**:13292 - 13301.

R&D Systems, Inc.
1-800-343-7475

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- AEBSF (R&D Systems, Catalog # EI001)
- Trypsin (Sigma, Catalog # T-1426)
- Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(DNP)-NH_2 (R&D Systems, Catalog # ES002), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Prepare a curve of AEBSF in Assay Buffer. Make the following serial dilutions: 10000, 2000, 500, 250, 150, 50, 25, 10, 2, and 1 µM.
2. Dilute Trypsin to 0.08 µg/mL in Assay Buffer.
3. Gently mix 30 µL of each of the E-64 curve dilutions with 30 µL of the 0.08 µg/mL Trypsin. Include a control (in duplicate) containing 30 µL Assay Buffer and 30 µL of the 0.08 µg/mL Trypsin.
4. Incubate mixtures at room temperature for 15 minutes.
5. Dilution of reaction mixture by adding 90 µL Assay Buffer to each reaction.
6. Dilute Substrate to 20 µM in Assay Buffer.
7. Load 50 µL of the incubated mixtures into a black well plate and start the reaction by adding 50 µL of 20 µM Substrate.
8. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration (IC_{50}) for AEBSF by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
10. The specific activity for Trypsin at each point may be determine using the following formula (if needed):

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor**(pmole/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions Per Well

Trypsin: 0.0008 µg
AEBSF curve: 1000, 200, 50, 25, 15, 5, 2.5, 1, 0.2, and 0.1 µM
Substrate: 10 µM