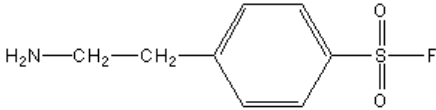


AEBSF

Product Description

A very effective serine protease inhibitor for biochemistry and biotechnological applications.
Better than DMP and PMSF and not toxic!

Chemical name :	4-(2-aminoethyl)-benzene-sulfonyl fluoride	 <p style="text-align: center;">• HCL</p>
Structure :	C ₈ H ₁₀ NSO ₂ F.HCl, MW: 239.7	
Toxicity :	LD50 : 2834 mg/kg	
Inhibitory activity :	$K_{app} / [I] \text{ (L.Mol}^{-1} .s^{-1})$	
	Trypsin : 14.00 Chymotrypsin : 18.70 Plasmin : 0.36 Thrombin : 1.62 Plasmatic Kallikrein : 0.68 Glandular Kallikrein : 0.19	
Storage : (L)	in a closed container, protected from moisture, at 4°C	

Benefits

very active (reacts at low concentrations, in low molar to molar ratios)
broad specificity and high affinity for serine proteases
reacts irreversibly with proteases and has only very little effect on other components of buffers and cell culture
non toxic, hence easy to handle
easily removed by dialysis
protect valuable therapeutic proteins

Applications:

downstream purification (chromatography, dialysis...)
affinity labelling of serine proteases
protection of proteins and peptides in extracts, purification process, storage...
manufacture of diagnostic proteins
recombinant protein cultures

Samples

animal cell extracts (0.4-2mM)
vegetal cell extracts
bacterial and fungi extracts
media and buffers
cell culture media (0.1-0.25mM)

Scientific and Technical Information

• solubility

AEBSF is a white crystalline solid from sulfonyl fluorides family, molecular weight 239, with a melting point of 180-182°C. It is **readily soluble in ethanol, in water and in aqueous buffer**, while most widely inhibitors DFP and PMSF have limited solubility or require anhydrous organic solvents. It takes less time to solubilize and does not precipitate out of solution when added to aqueous buffers (PMSF does), allowing

to use lower concentrations, that are kept more stable during process steps and storage.

Inhibitor	solubility (mg/ml)	
	water	Alcohol
AEBSF	200	75.0
PMSF	0.12 (decreased at high ionic strength)	10.5
DFP	15.4	20.0

• stability

AEBSF has an **excellent stability**, compatible with cell culture and downstream purifications.

For any question,
contact your local distributor

Uptima,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

uptima@interchim.com

The stability, maximal in water (pH5.2), is more or less decreased at higher temperatures, in alkaline conditions (pH>7) and with some solutes. Hydrolysis occurs upon reaction with hydroxyl ions above pH7.5. For maximum stability and reproducibility, it is thus recommended to store AEBSF in distilled water at 4°C where it is fully stable for up to 6 months, then to add it to buffer systems just before or during contact with biological materials, and to adjust pH just before use.

Now, the stability in aqueous buffer is sufficient without these precautions for most applications, eliminating the need (i.e. for PMSF) for frequent additions of inhibitor to multistep protein preparations. 70% of AEBSF remains after 22 hours at 4°C in phosphate buffer, 50% after 6 hours at 37°C. Experiments have shown with trypsin that the inhibitory activity of AEBSF is undiminished for at least 3 months at room temperature.

- **Activity**

In general, AEBSF demonstrates more effective effects than DFP, PMFS as general irreversible inhibitor of serine proteases (Walsman 1972, Markward 1974). AEBSF reacts with several serine proteases (plasmin, thrombin, kallikreins) with faster rates than either DFP and PMFS, and similar rates for trypsin and chymotrypsin.

Note: one should consider AEBSF induce modifications of protein, i.e. for 2D electrophoresis and can so potentially affect the isoelectric point of proteins.

reaction rates ($k_2/k_1 \text{ L}^{-1} \text{ M}^{-1} \text{ S}^{-1}$)

Enzyme	AEBSF	PMFS	DFP
Trypsin	3.06	2.57	6.23
Chymo trypsin	17.8	25.00	39.00
Plasmin	0.32	0.05	0.19
Thrombin	5.12	1.95	1.28
Plasma Kallikrien	0.68	0.07	0.30
Glandular Kallikrein	0.19	0.05	0.05
TPA	1.19	nd	nd
Subtilisin A	0.46	nd	nd

(Mintz 1993)

- **Toxicity**

AEBSF is a **user-friendly** reagent: In preliminary experiments, it was of great interest to determine the relative toxicity of protease inhibitors. In whole animal experiment with oral feeding, AEBSF is shown 354 times less toxic than DFP, and 14 times less toxic than PMFS. Additional studies determined LD50 of 0.4mg/kg in rabbits, whereas AEBSF has an LD50 of 76mg/kg when given to mice intravenously. As a result, that is also non toxic for cells in culture, allowing new applications. No inhibition of cell viability was evident up to a level of 0.25mM AEBSF, whereas a significant inhibition (60%) occurred at a level of 1mM (but this didn't increase at 4mM) and cells supplied with fresh serum started to proliferate once again.

As a result, AEBSF suits cell culture, added directly in media at low concentrations 0.1-0.25mM. It avoids for example the degradation of recombinant proteins secreted in culture by tissue culture cells or bacteria, prior to removal of the cells and purification and isolation of the product.

Other Information

Literature

- Mintz R.G., An Reversible Serine Protease Inhibitor, *Biopharm* Vol.6. N°2, March 1993, Pages 34-38
- Walsman P., M. Richter, and F.Markward, Inaktivierung von Trypsin and Thrombin durch 4-amidinobenzoylsulfofluoride, *Acta. Biol. Med. Germ.* 1972, 28. 577-585
- Markward F., P. Walsmann and Hoffmann J., Über den Einfluss Synthetischer Proteinase-inhibitoren auf die Wirkung und Aktivierung Proteolytischer Enzyme des Pankreas, *Acta. Biol. Med. Germ.* 1974, 32, 433-439
- James G.T., Inactivation of the Protease Inhibitor Phenylmethylsulfonyl Fluoride in Buffers, *Anal. Biochem.* 1978, 86, 574-579
- Depienne C., Mousnier A., Leh H., Le Rouzic E., Dormont D., Benichou S. and Dargemont C. ; Characterization of the Nuclear Import Pathway for HIV-1 Integrase ; *The Journal of Biological Chemistry* ; 2001; **276**:21, 18102-18107
- Laloux V., Beaudoin L., Jeske D., Carnaud C., and Lehuen A. ; NKT Cell-Induced Protection Against Diabetes in V&14-J&281 Transgenic Nodose Diabetic Mice Is Associated with a Th2 Shift Circumscribed Regionally to the Islets and Functionally to Islet Autoantigen ; *The Journal of Immunology*, 2001, **166**, 3749-3756
- Turpin P., Hay R.T., and Dargemont C. ; Characterization of IκBα Nuclear Import Pathway ; *The Journal of Biological Chemistry* ; 1999 ; **274**:10, 6804-6812
- Possot O.M., Gérard-Vincent. M, and Pugsley A. P. ; Membrane Association and Multimerization of Secretory Component PulC ; *Journal of Bacteriology* , 1999 ; **181**:13, 4004-4011

Rev.B09E

For any question,
contact your local distributor

Uptima,
powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

uptima@interchim.com