Uptima

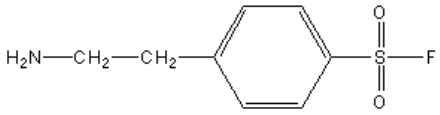
FT-401070

AEBSF

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

Product Description

AEBSF

Chemical name:	4-(2-aminoethyl)-benzene-sulfonyl fluoride		
Structure :	$C_8H_{10}NSO_2F.HCl$, MW: 239.7		
Toxicity:	LD50 : 2834 mg/kg		
Inhibitory activity:	K_{app} / [I] ($L.Mol^{-1}.s^{-1}$)		
	Trypsin : 14.00	Chymotrypsin : 18.70	Plasmin : 0.36
	Thrombin : 1.62	Plasmatic Kallikrein : 0.68	Glandular Kallikrein : 0.19
Storage :	in a closed container, protected from moisture, at 4°C		

Benefits	*Applications:*	*Samples*
very active (reacts at low concentrations, in low molar to molar ratios)	downstream purification (chromatography, dialysis…)	animal cell extracts (0.4-2mM)
broad specificity and high affinity for serine proteases	affinity labelling of serine proteases	vegetal cell extracts
reacts irreversibly with proteases ans has only very little effect on other components of buffers and cell culture	protection of proteins and peptides in extracts, purification process, storage…	bacterial and fungi extracts
non toxic, hence easy to handle	manufacture of diagnostic proteins	media and buffers
easily removed by dialysis	recombinant protein cultures	
protect valuable therapeutic proteins		cell culture media (0.1-0.25mM)

- **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 aquous buffer**, while most widely inhibitors DFP and PMSF have limited solubility or require anhydrous organic solvants. It takes less time to solubilize and does not precipitate out of solution when added to aquous buffers (PMFS 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 ioninc strenght)	10.5
DFP	15.4	20.0

Related products:
Other protease inhibitors (Aprotinine UP185582, PMSF #UP147376)

- **stability**

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

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 aquous 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 generals, AEBFS demonstrates more effective effects than DFP, PMFS as general irreversible inhibitor of serine proteases (Walsman 1972, Markward 1974) . AEBSF reacts with several serin 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. fo r 2D electrophorosis and can so potentially affect the isoelectric point of proteins.

reaction rates ($k2/k1\ L^{-1}\ M^{-1}\ S^{-1}$)

Enzyme	AEBSF	PMFS	DFP
Trypsin	3.06	2.57	6.23
Chymotrypsin	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 preliminar experiments, it was of great interest to determine the relative toxicity of proteases inhibitors. In whole animal experiment with oral feeding, AEBSF is shown 354 times less toxic than DFP, and 14 times less toxic than PMFS. Additionnal 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 inculture, allowing new applications. No inhibition of cell viability was evident up to a level of 0.25mM AEBSF, whereas a significant inhibition (60%) occured at a level of 1mM (but this didnt increased 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.

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Other Information

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