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TECHNICAL INFORMATION

Catalog Number: 158837
Protease Inhibitor Cocktail Kit

Introduction
The presence of undesired proteases during the isolation and purification of intact peptides and proteins usually corresponds to greatly reduced yields of pure and active proteins. This is particularly true for proteins isolated from cellular extracts, since many cells and microorganisms secrete proteolytic enzymes when grown *in vitro*. The need for effective protease inhibition is therefore essential for proper isolation of purified proteins.

Mode of Action
Proteases are enzymes which direct their action upon proteins, cleaving their targets into smaller fragments consisting of polypeptides, peptides and amino acids. There are a myriad of proteases active against practically all proteins. Some proteases are quite specific, while others cleave many different proteins.
(please see the MP Protease Inhibitors brochure for further general information on proteases).

Inhibitor Cocktails
Since there are such a large number of proteases, both specific and non-specific, as endo- and expo-peptidases, the selection of a satisfactory protease inhibitor requires careful choice. No one protease inhibitor is likely to be sufficiently effective against all proteases. As a result, it is desirable to have a ready source of protease inhibitors from which to select one that will perform under the given conditions of a specific project. Yet, often more than one contaminating protease may be present , so a single inhibitor, even when carefully selected , any not be capable of inhibiting all proteolytic activity. Consequently , the practice of preparing an inhibitor "Cocktail" of several protease inhibitors seems to be gaining favor in some areas of protein research. Again, in order to prepare an inhibitor cocktail a variety of different inhibitors is highly desirable.
A broad spectrum protease inhibitor cocktail should cover both endopeptidases and exopeptidases , non-specific and specific. The most commonly found protease classes include serine proteases, metalloproteases and acid proteases. A suitable multi-purpose cocktail must contain these three classes and a broad range inhibitor for all other areas . MP has developed a broad spectrum cocktail which covers all the above requirements. The cocktail consists of four major protease inhibitors: AEBSF, Sodium EDTA, Leupeptin and Pepstain A. The specificities and working concentration of these enzymes is detailed below.

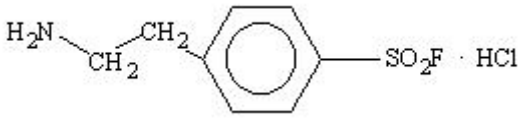
MP Protease Inhibitor Cocktail Kit
MP's Protease Cocktail Kit includes the following products in preweighed vials to prepare 100 ml of inhibitor cocktail:

Product	Catalog Number	Amount	Molar concentration (if prepared to 100 ml)	Typical Working Concentration
AEBSF Hydrochloride	193503	40 mg	1.67 mM	50-400 mg/ml
EDTA disodium dihydrate	195173	500 mg	13.4 mM	0.2-25 mg/ml
Leupeptin Hemisulfate	151553	0.1 mg	2.1 uM	0.5-1 mg/ml
Pepstatin A	195368	0.1 mg	1.45 uM	0.5-1 mg/ml

Preparation Instructions
Place the contents of all four (4) vials in one volumetric flask or other suitable container. Add water or buffer of choice to 100 mls. More concentrated solutions can be prepared as desired; however, leupeptin and pepstatin A will create more of a suspension at concentrations other than the suggested amount.
The prepared solution is stable for 60-90 days when stored at 5°C. Do not freeze the prepared solution, and avoid repeated freeze-thaw cycles.

Recommended Usage
One ml of the cocktail solution is recommended for the inhibition of proteases equivalent to 1 mg of USP pancreatin. One kit is recommended for the inhibition of proteases present in a maximum of 20 g of cell extract. Since not all organisms contain the same amount of endogenous proteases it may sometimes be necessary to increase the concentration of inhibitors.
Typical use is 1 ml of cocktail per 1 ml of sample.

Component Information
AEBSF
Structural formula:



Chemical formula: $C_6H_{10}FNO_2S$ HCl
Molecular weight: 239
Synonyms: p-Aminoethylbenzenenesulfonyl fluoride; 4-(2-Aminoethyl)-Benzenesulfonyl Fluoride Hydrochloride
Appearance: Slightly yellowish powder
Melting Point: 175º-177ºC
HPLC Purity:~ 97%
Description: Serine protease are proteolytic enzymes, characterized by a reactive serine residue, which participates in the caralytic mechanism.Trypsin-like proteases are those enzymes that attack substrates at peptide bonds, following an Arg or Lys residue.
AEBSF belongs to the family of irreversible sulfonyl fluoride inhibitors that block trypsin and chymotrypsin type enzymes. Similar in structure to the commonly used inhibitor PMSF. AEBSF form sulonyl derivatives of the active site serine residue.
The lower MW and toxicity of AEBSF relative to PMSF and related organophosphate inhibitors, make this inhibitor well suited for blocking trypsin-like activities in tissue culture media, as well as in large scale purification of recombinant proteins , susceptible to proteolysis.
AEBSF offers better solubility in water, stability, inhibitory activity, toxicity and handling than do other general inhibitors in use today (PMSF, DFP and benzomidine). AEBSF is very stable at neutral pH. IT shows only a slight hydrolysis at mildly alkaline pH's of 8-9. In comparison, DFP and PMSF hydrolyze relatively quick.
AEBSF is an excellent alternative to presently acailable general serine protease inhibitors; it offers superior inhibitory activity and much lower toxicological risk. It lends itself to use in a wide range of biological purification operations.
Characteristics
The presence of serine proteases in the preparative purifiaction and isolation of proteins and peptides usually causes the amounts of active, pure materials to be greatly reduced. In order to eliminate thse degradative proteolytic processes, a general serune inhibitor is desirable. Ideally, it should be added during the purificaiton to nullify or at least greatly reduce the activity of the proteases.
Such an inhibitor should possess the following characteristics :

– It should be easily soluble in water and stable at neutral pH.

– Its inhibitory activity should be strong and broad enough to effectively eliminate as many as serine protease as possible in the crude material.

– Its toxicity should be as low as possible so that normally skilled laboratory technicians can handle it saely and securely.

– The delivery of the inhibitor to the system under purification should be as standaridized and simple as possible.

Table 1
Relatively Toxicity of Inhibitors

Inhibitor	*LD50 * (MG/KG)*	*Relative Ratio*
AEBSF	2834	354
PMSF	200	25
DFP	8	1

* Oral administration

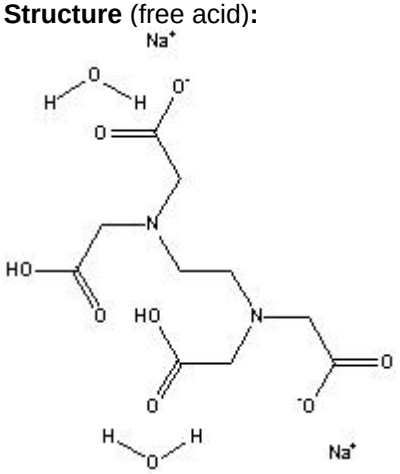
AEBSF was found to be approximatey 350 fold less toxic than DFP (LD_{50} dose required is in the order of 350X higher)

Table 2
Comparitive Rate Constants for Serine Protease Inhibitors

Enzyme	*AEBSF*	*PMSF*	*DFP*
Trypsin	14.00	2.57	6.23
Chymotrypsin	18.70	25.0	39.00
Plasmin	0.36	0.05	0.19
Thrombin	1.62	1.95	1.28
Pl. Kallikrein	0.68	0.07	0.30
Gl. Kallikrein	0.19	0.05	0.05

As can be seen from the above data, AESBF inhibits at a rate that is faster or comparable to other general inhibitors in use today.

EDTA, Disodium Salt, Dihydrate
Structure (free acid)**:**



Molecular Formula: $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$
Molecular Weight: 372.2
CAS # 6381-92-6

Physical Description: White Powder
Synonyms: EDTA; Ethylenedinitrilotetraacetic acid; Versene; [Ethylenedinitrilo] tetraacetic acid
Description: EDTA is a divalent cation chelator used in some electrophoresis buffer systems. Some proteolytic enzymes and nucleases are divalent cation dependent. The addition of EDTA reduces the chance of sample degredation. Used to eliminate inhibition of enzyme catalysed reactions due to traces of heavy metals. The addition of EDTA may interfere with metal ion-dependent biological processes. A reversible metalloprotease inhibitor (Effective concentration is typically 1-10 mM).
References:

– *Merck Index*, **12th Ed.**, Nos. 3556, 3557, 3558, 3559.
– Powers, J.C. and Harper, J.W., *Proteinase Inhibitors*, (Barrett, A.J. and Salvesen, G., eds) Amsterdam, Elsevier, p. 219 (1986).
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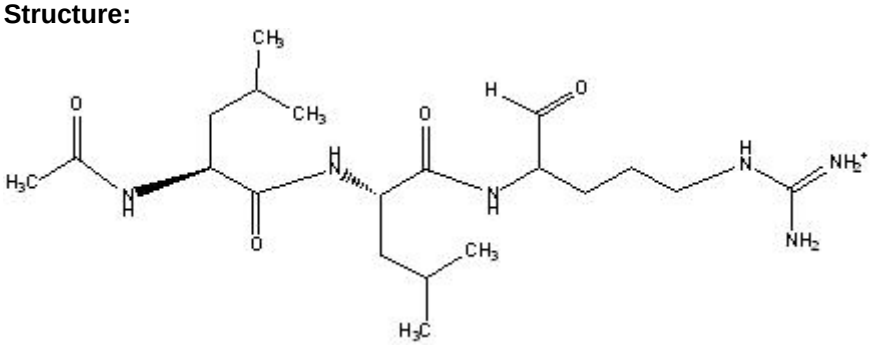
Leupeptin Hemisulfate

Structure:



Molecular Formula:
$C_{20}H_{38}N_6O_4 \cdot ½H_2SO_4$
Molecular Weight:
475.6
CAS #
103476-89-7

Synonym: Acetyl-Leu-Leu-Arg-al
Physical Description: White to off-white powder
Description: A modified tripeptide reversible protease inhibitor of trypsin-like proteases and some cysteine proteases[12] including endoproteinase Lys-C, kallikrein, papain, thrombin, cathepsin B[6], cathepsins H and L[14], trypsin, calpain[13], trypsin[8] and plasmin. Effective concentration is typically 10-100 uM. Little to no inhibition is seen against pepsin, cathepsins A and D and alpha-chymotrypsin. When adjusted for molarity, all three salt forms are equally effective; however, the hydrochloride salt is usually less invasive in biological settings.
Leupeptin, because of its aldehyde group, may act as a reducing agent and therefore interfere in protein determinations such as Lowry and, to a lesser extent, Bradford.[9]
Note: Leupeptin gives multiple peaks on HPLC due to equilibria among three forms in solution. Purity determined by HPLC should take into account the three main peaks.[11] Majority of contaminating peptide is racemized leupeptin.
The primary mechanism of inactivation is racemization of the L-arginal; the D-arginal form is totally inactive. If the aldehyde is oxidized but retains its L-configuration, the resulting compound does have some inhibitory activity.
References:

– *Merck Index*, **12th Ed.**, No. 5483.
– Aoyagi, T., *J. Antibiot.*, **v. 22**, 283 (1969).
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– Zimmerman, U.-J.P. and Schlaepfer, W.W., *Biochemistry*, **v. 21**, 3977 (1982).
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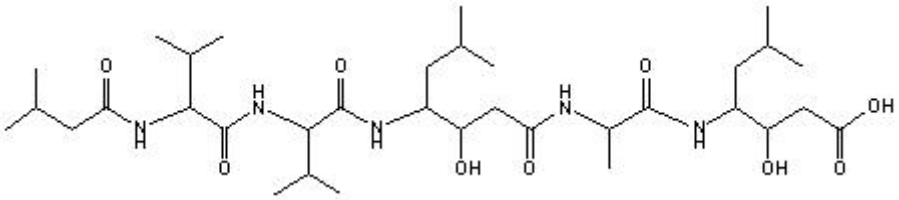
Pepstatin A

Structure:



Sequence: Isoval-Val-Val-Sta-Ala-Sta; where Sta = statine = (SS, 4S)- 4-amino-3- hydroxy-6- methylheptanoic acid.
Molecular Formula: $C_{34}H_{63}N_5O_9$
Molecular Weight: 685.9
CAS # 26305-03-3
Synonyms: Isovaleryl-L-valyl-L-valyl-[(3S, 4S)-4-amino-3-hydroxy-6- methylheptanoyl]-L- alanyl [3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid]; [1S-[1R*,2R*,4[R*[R*(R*)]]]]-N-(3-methyl-1-oxobutyl)-L-valyl-N-[4-[[2-[[1-(2-carboxy-1-hydroxyethyl)-3- methylbutyl] amino]-1-methyl-2-oxoethyl]amino]-2-hydroxy-1-(2-methylpropyl)-4-oxobutyl]-L-valinamide; N-isovaleryl-L-valyl-L-valyl-3-hydroxy-6-methyl-g-aminoheptanoyl-L- alanyl-3-hydroxy-6-methyl-g-aminoheptanoic acid
Source: Synthetic
Description: A reversible inhibitor of aspartic proteases. Inhibitor for pepsin, renin, cathepsin D, cathepsin G, and other acid proteases. It does not inhibit thiol proteases, neutral proteases or serine proteases. Pepstatin forms a 1:1 complex with acid proteases (carboxyl proteases). Effective concentration is approximately 1 uM (0.5 to 1.0 ug/mL).
Click Here for a list of other protease inhibitors offered by MP Biomedicals and general protease inhibitor information.
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