Product Information

MS-SAFE Protease and Phosphatase Inhibitor

Mass Spectrometry Safe Protease and Phosphatase Inhibitor Cocktail

MSSAFE

Product Description

Crude cell extracts contain a myriad of endogenous enzymes, such as proteases and phosphatases, which can degrade or modify the proteins present in the sample. The best way to preserve the integrity of the proteins is to add a broad spectrum of protease and phosphatase inhibitors tailored to the sample and task.

MS-SAFE is a mass spectrometry-compatible, general purpose protease and phosphatase inhibitor cocktail. The protease inhibitors in MS-SAFE have broad specificity for the inhibition of serine, cysteine, aspartic, and metalloproteases. The phosphatase inhibitors in MS-SAFE act upon tyrosine, serine/threonine, acid and alkaline phosphatases. MS-SAFE contains a unique formulation, including a pepstatin A salt, to provide additional protection not normally obtained in a cocktail format.

All inhibitors, plus the fillers, were selected so as not to interfere with LC-MS analyses. Any inhibitors capable of covalent, irreversible protein modification were avoided. For example, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), a commonly used potent serine protease inhibitor, has been demonstrated to modify non-target proteins, and thus introduce artifacts in mass spectral interpretation.[1] Therefore, AEBSF is not used in MS-SAFE.

MS-SAFE is EDTA-free. All inhibitors were selected to allow facile downstream sample processing, such as immobilized metal affinity chromatography (IMAC) for His-tagged protein purification and, uniquely, phosphopeptide enrichment. Of note, most phosphatase inhibitor cocktails contain glycerophosphate, molybdate, or other compounds known to compromise phosphopeptide enrichment using iron, gallium, or titanium dioxide-based affinity matrices.[2]

MS-SAFE has been tested in mammalian cell lysates and liver tissue extracts. Several theses[12] and dissertations[13-27] have cited use of product MS-SAFE in their research protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Vials of MS-SAFE powder are stable, as supplied, for at least 2 years at 2-8 °C.

A 10× concentrated solution of MS-SAFE was tested and found to be stable for 16 hours on ice, or for 4 days when stored frozen at −20 °C. Avoid repeated freeze/thaw cycles of the frozen 10× solution.

Components

Each vial of MS-SAFE can be used to prepare 20 mL of 1× inhibitor solution, which contains the following inhibitors. Inhibitory targets of the components are shown:

- Bestatin hydrochloride (Cat. No. B8385): aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase[3,4]
- Leupeptin (Cat. No. L2884): serine and cysteine proteases, such as trypsin, plasmin, trypsinogen, urokinase, and kallikrein[3,4]
- Phosphoramidon disodium salt (Cat. No. R7385): thermolysin and collagenase[3]
- Pepstatin A (Cat. No. P5318): acid proteases, such as pepsin, renin, and cathepsin D, and many microbial aspartic proteases[3]
- Elastatinal (Cat. No. E0881): elastase[3,4]

- Aprotinin (Cat. No. A3428): serine proteases, such as chymotrypsin, trypsin, and elastase[5,6]
- Nafamostat mesylate (Cat. No. N0289): serine proteases, kallikrein
- Antipain (Cat. No. A6191): serine/cysteine proteases and some trypsin-like serine proteases[3]
- Okadaic Acid (Cat. No. O7885): type 2A protein phosphatases[8]
- Sodium Fluoride (Cat. No. S7920): serine and threonine phosphatases[9]
- Sodium Orthovanadate (Cat. No. S6508): ATPases, protein tyrosine phosphatases and other phosphate-transferring enzymes[10]
- Bromotetramisole Oxalate (Cat. No. 190047): L-isoforms of alkaline phosphatases[11]

β-Lactose & DL-Leucine are added as filler.

Preparation Instructions

A 1× working solution of MS-SAFE is prepared by adding 20 mL of extraction/lysis buffer to one vial of powder.

Alternatively, a 10× concentrated solution of MS-SAFE may be prepared by adding 2 mL of water or extraction/lysis buffer to one vial of powder. This 10× solution may then be diluted 10-fold into extraction/lysis buffer as needed for a 1× working solution.

Prior to use, the 1× or 10× solution should be mixed (15 minutes) until dissolved. Concentrations greater than 1× may appear slightly hazy. This will not affect the performance of the inhibitors.

Procedure

MS-SAFE has been tested for inhibition of proteases and phosphatases in mammalian samples. 20 mL of a 1× working solution of MS-SAFE in CelLytic™ M (Cat. No. C2978) mammalian cell lysis buffer will inhibit proteases and phosphatases present in a HeLa cell extract prepared from 2 × 10^8 cells (60 mg total protein by BCA). 20 mL of a 1× MS-SAFE working solution will inhibit proteases and phosphatases in the extract from 15 mg of rat liver.

Since all organisms will contain different types and amounts of endogenous proteases and phosphatases, it may be necessary first to test MS-SAFE for effectiveness in non-mammalian samples.

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