ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

PMSF (phenylmethanesulphonylfluoride or phenylmethylsulphonyl fluoride, Serine Protease inhibitor

Cat. # PMSF16-S-50	PMSF (phenylmethanesulphonylfluoride) 100X stock solution (0.1M)	**SIZE**: 50 ml
Cat. # PMSF16-S-250	PMSF (phenylmethanesulphonylfluoride) 100X stock solution (0.1M)	**SIZE**: 250 ml

Most researchers study expression or changes in a given protein in various cultured cells or tissues from animal or human origin. The first step is to harvest the proteins by disrupting the cells (lysis) and homogenization and extraction of cellular proteins. Many methodologies including homogenization, centrifugation and sonication have been employed to prepare cell or tissue extracts for further analyses by ELISA, Western, and IP etc. Many proteins are susceptible to rapid proteolysis or fragmentation of proteins after cell lysis or tissue disruption. Therefore, it is very important to minimize proteolysis and keep the phosphatase inactive.

RIPA (**R**adio-**I**mmuno**p**recipitation **A**ssay) Lysis Buffer is the most common buffer for rapid, efficient cell lysis and solubilization of proteins from both adherent and suspension cultured mammalian cells. It is widely used for cell lysis followed by immunoprecipitation (IP or co-IP) or direct western blotting. Most antibodies and protein antigens are not adversely affected by the components of this buffer. In addition, RIPA Lysis Buffer minimizes nonspecific protein-binding interactions to keep background low, while allowing most specific interactions to occur, enabling studies of relevant protein-protein interactions.

The RIPA Lysis Buffer components have protease inhibitors along with PMSF (a broad spectrum protease inhibitors). It can also be added in any buffer containing protein sor recombinant proteins.

PMSF (phenylmethanesulphonylfluoride or phenylmethylsulphonyl fluoride) is a serine protease inhibitor. However, it does not inhibit all serine proteases. PMSF is rapidly degraded in water and stock solutions are usually made up in anhydrous ethanol, isopropanol, corn oil, or DMSO. Proteolytic inhibition occurs when a concentration between 0.1 - 1 mM PMSF is used. The half-life is small in aqueous solutions (110 min at pH=7 and 35 min at pH=8).

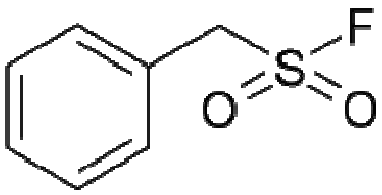
PMSF binds specifically to the active serine residue in a serine proteases. It does not bind to any other serine residues in the protein. This is a result of the hyperactivity of that serine residue caused by the specific environmental conditions in the enzymes active site. Because PMSF binds permanently to the enzyme, the complex can be viewed by X-ray crystallography, it can therefore be used as a chemical label to identify an essential active site SER in an enzyme.

Enzyme(active)SER-O-H + F-SO2CH2C6H5 --> EnzymeSER-O-SO2CH2C6H5 + H-F

Serine Protease + PMSF --> irrevsible Enzyme-PMS complex + Hydroflouride

The LD50 (lethal dose) is less than 500mg/kg. PMSF is a cytotoxic chemical which should be handled only inside a fume hood.

PMSF is commonly used in protein solublization in order to deactivate proteases from digesting proteins of interest after cell lysis.



PMSF: Mol wt =174.2 ($C_7H_7FO_2S$)

Purity: >99%, White to off-white crystalline solid

Solubility: Ethanol, isopropanol, or methanol

Storage: 15 to 30℃. Protect from moisture. Following reconstitution, refrigerate (4℃). Stock solutions are stable for up to 9 months at 4℃.

Effective Concn: 0.1-1 mM final concn.

Form: the product is supplied as ready to use solution (100X) in ethanol. If used at 1:100 then the final concn will be 1mM. Store solution at 4oC.

Suggested Usage: Use 10 ul/ ml or 1 ml per 100 ml of buffer or cell lysate or protein solutions. Add PMSF before use and do not store PMSF buffer for prolong period.

References : Seitz, R., et al. 1993. *Int. J. Cancer* **53,** 514. Weaver, V.M., et al. 1993. *Biochem. Cell. Biol.* **71,** 488. Bourgain, R.H., et al. 1992. *Adv. Exp. Med. Biol.* **316,** 427. Chang, C.T., et al. 1992. *Biochem. Int.* **28,** 707

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