

## 10×Protease and Phosphatase Inhibitor Cocktail Mix

**Cat:** IKM1020

**Storage:** Store at -20°C, 1 year.

### Introduction

Extracts such as cells or tissues contain many endogenous proteases, phosphatases, etc. In the in vitro environment, the proteins in the extracts are prone to degradation or modification, which affects subsequent protein detection. Therefore, adding inhibitors such as proteases and phosphatases to the extract is an effective method to prevent protein degradation and demodification.

10 × Protease and Phosphatase Inhibitor Cocktail is a mixture of universal protease and phosphatase inhibitors, which can effectively inhibit various proteases (such as serine protease, aminopeptidase, cysteine protease, threonine and aspartic protease, metalloproteinases, etc.) and phosphatases (such as serine / threonine, tyrosine, acid and alkaline phosphatase, etc.) in conventional cell or tissue extracts. Aprotinin is a competitive reversible inhibitor of serine protease; bestatin is a reversible inhibitor of aminopeptidase. E-64 is an irreversible inhibitor of cysteine protease; leupeptin is a reversible inhibitor of serine and cysteine proteases;  $\beta$ -Glycerophosphate is a reversible inhibitor of serine/threonine phosphatase; Sodium fluoride is a reversible inhibitor of acid phosphatase. Sodium orthovanadate is a reversible inhibitor of alkaline phosphatase and tyrosine phosphatase. Sodium pyrophosphate is an irreversible inhibitor of serine / threonine phosphatase. It can be widely used in WB, Co-IP, pull-down, IF, IHC, kinase assay and so on.

This product is presented with an additional 100 mM EDTA, and customers can choose whether to use it according to their own needs.

### Kit Components

Composition	Component	Solvent	Size	Storage
10×Protease and Phosphatase Inhibitor Cocktail (Universal type)	0.08 mM Aprotinin	Water	1*1mL	-20°C
	5 mM Bestatin			
	1.5 mM E-64			
	2 mM Leupeptin			
	10 mM $\beta$ -Glycerophosphate			
	50 mM Sodium fluoride			
	1 mM Sodium orthovanadate			
	10 mM Sodium pyrophosphate			
100 mM EDTA	100 mM EDTA	Water	1*1mL	2-8°C

### Product advantage

1. The product ingredients are fully open and the concentration is clear
2. Good product compatibility, efficient inhibition of a variety of proteases, comprehensive protection from protein degradation

3. All products in this series are provided with an additional 100 mM EDTA, which can be used directly without additional preparation
4. Flexible customization. Customers can customize the desired MIX for their own experiments or customize the exclusive Kit for a single component. Custom specifications and packaging are also available.

**Protocols** (*only for reference*)

1. Before use, the product should be thawed at room temperature, and centrifuged at a low speed before opening the lid, so as to better shake the liquid adhered to the pipe wall to the bottom of the pipe.
2. The inhibitor mixture was added to the solution sample (such as cell lysis solution or tissue extract) at a ratio of 1 : 10 and used after mixing.
3. The product can be properly subpackaged and stored, and the lysis solution containing the inhibitor mixture should be used now, and should not be used after cryopreservation.
4. If you need to use EDTA, you can add it to the cracking solution in an appropriate proportion and use it after mixing.

**Note**

1. If the subsequent experiments need to detect the activity of metalloproteinases in the extract, EDTA should not be added.
2. This product is only for scientific research. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics.
3. For your safety and health, please wear experimental clothes and wear disposable gloves and masks.

**FAQ**

Q : Can Inhibitor Cocktail not be diluted according to the specified proportion?

A: Our Cocktail uses a classic concentration ratio. In order to ensure that it has sufficient inhibitory effect on different types of proteases, it is recommended to dilute it in accordance with the specified ratio. However, the inhibitory effect of protease activity is related to many factors, such as the difficulty of target protein degradation, the concentration of protease, the activity of protease and the concentration of inhibitor, which will affect the final protective effect. The content of phosphatase in samples from different sources is different. Therefore, in actual use, the concentration can be adjusted appropriately according to the experimental results.

Q : Why use the inhibitor Cocktail? What are the advantages compared with the commonly used PMSF?

A : Cocktail is a low-toxic, comprehensive protein protection reagent that maximizes the protection of proteins from degradation by proteases. More reliable than a single inhibitor. PMSF is a classical serine protease inhibitor, which is widely used in the process of cell lysis and purification of proteins. However, PMSF has obvious shortcomings in many aspects, one

of which is high toxicity. Therefore, AEBSF is selected as its substitute in protease inhibitors. The second is easy degradation failure, which is easy to fail during cell lysis, and the protease inhibitor Cocktail not only protects more comprehensively, but also has a more lasting effect.

Q: Will DMSO affect the experiment?

A: In general, DMSO does not affect the experimental results. DMSO is also used as a solvent for many bioactive substances, such as protein crystallization and co-immunoprecipitation. DMSO is stable at room temperature, and DMSO is also an excellent cell cryoprotectant at low temperature.

Q: I used Cocktail, but the results are still not ideal. Why?

A: For most proteins, Cocktail has a good effect. If there is still a low protein yield, it is necessary to carefully check the experimental steps and program design to reduce the links leading to protein degradation. For example, before cell treatment, the lysis system should be fully prepared, and Cocktail should be added in advance and mixed well. The cells to be broken should be added to the prepared lysis solution immediately after collection or removal from the refrigerator, so as to ensure the whole low temperature operation. If the target protein is special, there is no inhibitor of a specific protease family in the Cocktail, and a specific protease inhibitor can be added to the lysate.