

## PMSF Protease Inhibitor

Catalog No : E-EL-SR002

Product size: 10mL

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

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## Use

The reagent is used to inhibit protease activity released by broken cells during cell extraction/cell lysate/tissue homogenate sample collection in ELISA experiments.

## Storage

The reagent can be stably stored at 2-8°C for 6 months.

## Background

Breaking the cell to extract proteins releases proteases that need to be rapidly inhibited to keep the protein from being degraded. Therefore, protease inhibitors should be added before the cells break down to prevent proteolysis during protein extraction. PMSF protease inhibitor inhibits serine proteases (e.g., chymotrypsin, trypsin, thrombin) and sulfhydryl proteases (e.g., papain).

## Concentration

Product concentration: 10mg/mL

Effective working concentration: 17-174µg/mL

## Method of use

Tissue homogenate:

1. The tissue was washed with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood or other impurities on the surface. After weighing, the tissue the tissues should be minced into small pieces.
2. Add an appropriate amount of protease inhibitor to pre-cooled PBS(effective concentration of inhibitor is 17-174µg/mL), and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles.
3. The homogenates are centrifuged for 5-10 min at 5000×g at 2-8°C. Then collect the supernatant, and immediately use it for ELISA tests or store in aliquots at -20°C/-80°C, avoiding repeated freeze-thaw.

Cell extraction/cell lysis fluid:

1. Aspirate the medium in the culture plate, digest the cells with trypsin (EP-CM-L0043), and prepare cell suspension. This operation is omitted for suspended cells.
2. The cell suspension was collected into the centrifuge tube and centrifuged at 2-8°C for 5 minutes at 1000×g, then discard the medium and wash the cells 3 times with pre-cooled PBS.
3. Add an appropriate amount of pre-cooled PBS (add protease inhibitor before use, effective concentration 17-174µg/mL) to resuspend the cells. Recommendation: Add 150-250µL pre-cooled PBS to each well (about  $1 \times 10^6$  cells) of 6-well culture plate to resuspend the cells.
4. Repeat the freeze-thaw process several times or use an ultrasonic cell disrupter until the cells are fully lysed.
5. Centrifuge for 10 min at 1500×g at 2-8°C to collect the supernatant, and immediately use it for ELISA tests or store in aliquots at -20°C/-80°C, avoiding repeated freeze-thaw.

### **Notes for samples**

Samples should be assayed within 7 days when stored at 2-8°C, otherwise samples must be divided up and stored at -20°C ( $\leq 1$  month) or -80°C ( $\leq 3$  months). Avoid repeated freeze-thaw cycles.

### **Note**

1. This product is proved to be used for ELISA sample collection, and it is recommended to use it with ELISA kit products provided by our company. For other experimental purposes, scientific research users need to consult relevant information to explore, our company is not responsible for the relevant experimental operations and experimental results.
2. This product is toxic to human body, please be very careful when handling it, pay attention to effective protection, avoid direct contact with human body or inhalation.
3. PMSF is unstable in aqueous solution and should be used and added immediately before lysis of tissues or cells.
4. The product is designed for research use only, not for clinical diagnosis and treatment.