Protocol

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Mammalian Protease Inhibitor Cocktail Preparation Protocol

To prevent protein degradation during cell lysis

Introduction

Protease inhibitors are used to inactivate enzymes called proteases which degrade proteins. During cell lysis, the contents of the cell are released which includes many different proteolytic enzymes. An important component of any cell lysis protocol includes the addition of protease inhibitors to protect the integrity of the cellular proteins. In general, protease inhibitors are added individually to the desired concentration or as a mixture of predefined inhibitors, commonly referred to as a "protease inhibitor cocktail". In order to provide the investigator with maximum flexibility, GoldBio offers both individual protease inhibitors and preformulated protease inhibitor cocktails.

Benzamidine is a reversible competitive inhibitor of serine proteases. Leupeptin is a reversible competitive inhibitor of cysteine, serine and threonine proteases. Aprotinin is an irreversible inhibitor of serine proteases. Pepstatin A is a reversal inhibitor of aspartic proteases. EDTA and EGTA are both metalloprotease inhibitor and chelators. EDTA has a high affinity for magnesium ions, while EGTA has a high affinity for calcium ions. It is recommended to use at least one of these metalloprotease inhibitors during cell lysis unless downstream applications include immobilized metal affinity chromatography, PCR or other processes inhibited by metal chelators. AEBSF and PMSF are both irreversible serine protease inhibitors that also inhibit certain mammalian specific proteases. It is recommended to use at least one of them during lysis of mammalian cells. This protocol for mammalian protease inhibition is adopted from *Lysis of Cultured Cells for Immunoprecipitation* (Hong Ji 2010).

Materials

- Benzamidine Hydrochloride Monohydrate, GoldBio Catalog # B-050 [CAS 1670-14-0, mw. = 174.63 g/mol
- Leupeptin Hemisulfate, GoldBio Catalog # L-010 [CAS 103476-89-7, mw. = 475.59]
- Pepstatin A, GoldBio Catalog # P-020 [CAS 26305-03-3, mw. = 685.89]
- Aprotinin, GoldBio Catalog # A-655 [CAS 9087-70-1, mw. = 6511.44 g/mol]
- EDTA Disodium, GoldBio Catalog # E-210 [CAS 6381-92-6, mw. = 372.24 g/mol]
- AEBSF, GoldBio Catalog # A-540 [CAS 30827-99-7, mw. = 239.7]
- Mammalian Cell Lysis Buffer, GoldBio Catalog # GB-180

Optional:

- PMSF, GoldBio Catalog # P-470 [CAS 329-98-6, mw. = 174.19]

- EGTA, GoldBio Catalog # E-217 [CAS 67-42-5, mw. = 380.35]

Method

Sample Preparation

1. Obtain washed cell pellet of 1 x 10^7 to 5 x 10^7 cells.

2. Resuspend pellet in 1 ml of lysis buffer (see Mammalian Cell Lysis Buffer protocol).

Protease Inhibition

3. Add 10 µl of 100mM Benzamidine HCl (see Benzamidine HCl stock solution protocol) per 1 ml of lysis buffer.

4. Add 10 µl of 10mM Leupeptin Hemisulfate (see Leupeptin stock solution protocol).

5. Add 15 µl of 1mM Pepstatin A (see Pepstatin A stock solution protocol).

6. Add 1 µl of 10 mg/ml Aprotinin (see Aprotinin stock solution protocol).

7. Add 2 µl of 0.5M EDTA (see EDTA stock solution protocol) **OR** 0.5M EGTA stock solution.

8. Add 2 µl of 100mM AEBSF (see AEBSF stock solution protocol) **OR** 2 µl of 100mM PMSF (see PMSF stock solution protocol).

9. Mix by pipetting up and down and continue with cell lysis.

Tips

- This protease inhibitor cocktail can also be incorporated into a lysis buffer recipe by replacing equal volumes of water with the volumes of protease inhibitors for a 1 ml sample.

- The final working concentrations of each protease inhibitor are: 1mM Benamidine HCl, 100µM Leupeptin, 15µM Pepstatin, 1 µg/ml Aprotinin, 1mM EDTA or EGTA, and 200µM AEBSF or PMSF.

- **PMSF is toxic! WEAR GLOVES AND GOGGLES.**

References

Ji, H. (2010). Lysis of cultured cells for immunoprecipitation. *Cold Spring Harbor Protocols*, *2010*(8), pdb-prot5466.