Proti-Ace™ Kit
User Guide

Application
In situ proteolysis and proteolytic screening of protein samples for crystallization and structure determination

Features
- 6 proteases
- Stable, optimized, freeze dried protease formulation
- Enhanced stability
- Proti-Ace Dilution Buffer
- Optimized protocol for in situ proteolysis or proteolytic screening

Kit Contents
Proti-Ace Reagent 1* (Qty 3):
- 1 mg/ml α-Chymotrypsin,
  1 mM Hydrochloric acid,
  2 mM Calcium chloride dihydrate

Proti-Ace Reagent 2* (Qty 3):
- 1 mg/ml Trypsin,
  1 mM Hydrochloric acid,
  2 mM Calcium chloride dihydrate

Proti-Ace Reagent 3* (Qty 3):
- 1 mg/ml Elastase,
  200 mM Tris pH 8.0

Proti-Ace Reagent 4* (Qty 3):
- 1 mg/ml Papain,
  2 mM EDTA,
  2 mM TCEP hydrochloride,
  2 mM L-Cysteine

Proti-Ace Reagent 5* (Qty 3):
- 1 mg/ml Subtilisin,
  10 mM Sodium acetate trihydrate pH 7.5,
  5 mM Calcium acetate hydrate

Proti-Ace Reagent 6* (Qty 3):
- 1 mg/ml Endoproteinase Glu-C (in deionized water)

Proti-Ace Dilution Buffer (Qty 6):
- 1.6 ml of 10 mM HEPES pH 7.5,
  500 mM Sodium chloride

* When 100 μl of deionized water is added to each Proti-Ace freeze dried reagent

Discussion
A proteolytic fragment or domain of a protein may crystallize more readily or form better diffracting crystals than the intact protein. Proteases can be used to generate small, active fragments or domains of the target protein for crystallization. The fragment or domain can be used directly for crystallization experiments. Or the proteolytic sample analyzed by gel electrophoresis and/or mass spectrometry for mass and sequence for subsequent cloning, expression, purification and crystallization. Using proteolysis to enhance sample crystallization, the current overall success rate for yielding a deposited crystal structure is currently better than 12%.

Instructions for Proteolytic Screening
Proteolytic screening is a procedure involving limited proteolysis of the sample versus a portfolio of proteases, followed by denaturing gel electrophoresis (SDS-PAGE) and/or mass spectrometry (MS) to identify regions of a gene corresponding to the protease resistant domain/fragment for subsequent cloning, expression, purification and crystallization.

1. Select all six, a subset or a single protease from the Proti-Ace kit for Proteolytic Screening.
2. Add 100 μl of deionized water to each of the selected Proti-Ace enzymes to create a 1 mg/ml Protease Stock solution.
3. Into empty micro centrifuge tubes, create a 1:100 dilution (0.01 mg/ml) of each 1 mg/ml Protease Stock from Step 2 by adding 5 μl of the 1 mg/ml Protease Stock plus 495 μl of Proti-Ace Dilution Buffer (10 mM HEPES pH 7.5, 500 mM Sodium chloride).
4. Pipette 10 μl of the 1:100 protease stock into 10 μl aliquots of protein (10 mg/ml) for each protease to be screened.
5. Incubate at 37°C for 60 minutes.
6. Stop the reaction by adding SDS-PAGE sample buffer for SDS-PAGE analysis or a final concentration of 10% v/v trichloroacetic acid for MS analysis. Refer to your SDS-PAGE and MS protocols for the appropriate volume and concentration of SDS-PAGE or MS sample buffer for quenching.
7. Analyze the digests by SDS-PAGE and/or MS. Identify the small active fragment (SAF) or protease resistant domains. Clone the corresponding region of the gene. Express, purify and crystallize this gene product. Alternatively, scale up the proteolysis and purify the digest to produce a pure homogeneous sample of the SAF or domain for crystallization.

In the event of insufficient digestion, repeat steps 1-3 using a higher protease concentration such as 1:10 dilution of each Protease Stock (5 μl of 1 mg/ml Protease Stock plus 45 μl of Proti-Ace Dilution Buffer). Also consider longer incubation times, up to 24 hours.

In the event of over digestion, repeat steps 1-3 using a lower protease concentration such as 1:1,000 dilution of each Protease Stock (10 μl of the 1:100 Protease Stock plus 90 μl of Proti-Ace Dilution Buffer). Also consider shorter incubation times and/or lower incubation temperature (4 to 25°C).
Instructions for In Situ Proteolysis

In situ proteolysis is a procedure where trace amounts of protease are included with the sample to be crystallized and mixed with crystallization reagents for screening or optimization experiments.1-4

1. Select the desired protease(s) from the Proti-Ace kit to be used for in situ proteolysis.
2. Add 100 µl of deionized water to each of the selected Proti-Ace enzymes to create a 1 mg/ml Protease Stock solution.
3. Add the protease to the protein crystallization sample. Add 10 µl of the 1 mg/ml Protease Stock solution to 90 µl of 10 mg/ml protein to create a 1:100 w/w dilution.
4. Set the crystallization experiment using the protease:sample mixture.

Optimization of In Situ Proteolysis for Crystallization

a. Vary the protease:sample ratio. Typical protease:sample ratios are 1:100, 1:1,000 and 1:10,000.
b. Alter the incubation time. Typical incubation times are between 0 and 24 hours.
c. Alter the incubation temperature. Typical incubation temperatures are between 4 and 37°C.
d. For protein concentrations other than 10 mg/ml one can either use the preferred sample concentration with the protease:sample dilutions described in steps 1-4 or one can dilute the Proti-Ace 2 enzymes to a perfect 1:100 ratio based on the actual protein concentration. For example, if the protein concentration is 20 mg/ml one can add 50 µl of deionized water in step 2 to create a 2 mg/ml Protease Stock solution and then proceed with steps 3 and 4 to screen 1:100 protease:sample.

Storage of the Proti-Ace Kit

The unique freeze dried formulation of the Proti-Ace kit offers a much improved protease stability compared to liquid protease formulations. Recommended storage: Room temperature up to 30 days, 4°C up to 12 months, -20°C up to 24 months. Once the proteases are made into solution the recommended storage is: Room temperature to 4°C up to 24 hours, -20°C up to 12 months.

References


Related Products

HR2-429-01 Proti-Ace Reagent 1: 1 mg/ml α-Chymotrypsin, 1 mM Hydrochloric acid, 2 mM Calcium chloride dihydrate
HR2-429-02 Proti-Ace Reagent 2: 1 mg/ml Trypsin, 1 mM Hydrochloric acid, 2 mM Calcium chloride dihydrate
HR2-429-03 Proti-Ace Reagent 3: 1 mg/ml Elastase, 200 mM Tris pH 8.0
HR2-429-04 Proti-Ace Reagent 4: 1 mg/ml Papain, 2 mM EDTA, 2 mM TCEP hydrochloride, 2 mM L-Cysteine
HR2-429-05 Proti-Ace Reagent 5: 1 mg/ml Subtilisin, 10 mM Sodium acetate trihydrate pH 7.5, 5 mM Calcium acetate hydrate
HR2-429-06 Proti-Ace Reagent 6: 1 mg/ml Endoproteinase Glu-C
HR2-429-07 Proti-Ace Dilution Buffer: (10 mM HEPES pH 7.5, 500 mM Sodium chloride), 1.6 ml

* When 100 µl deionized water is added to the supplied freeze dried Proti-Ace reagent.