Proti-Ace™ Kit

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-429 (pg 1)**

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.[1-8] Proteases can be used to generate small, active fragments or domains of the target protein for crystallization.[9] 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%.[3]

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.[9] 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).

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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.[2-4]

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 and/or 1:1,000 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

1. Allan D'Arcy, personal communication, 1989-2009
2. In situ proteolysis for protein crystallization and structure determination. Dong, A et al. Nature Methods - 4, 1019 - 1021 (2007)
3. In Situ Proteolysis to Generate Crystals for Structure Determination: An Update. Amy Wernimont, Aled Edwards. PLoS ONE 4(4): e5094. doi:10.1371/journal.pone.0005094
4. The use of in situ proteolysis in the crystallization of murine CstF-77. Tong et al. Acta Cryst. (2007). F63, 135-138
5. A brief history of protein crystal growth. McPherson, A. Journal of Crystal Growth, vol. 110, issue 1-2, pp. 1-10, 1991
6. Preparation and analysis of protein crystals. McPherson, A. John Wiley & Sons, Inc. 1982. ISBN 089464355X
7. A crystallizable form of the Streptococcus gordonii surface antigen SspB C-domain obtained by limited proteolysis. Forsgren et al. Acta Cryst. (2009). F65, 712–714
8. Preliminary X-ray analysis of a human VH fragment at 1.8 angstrom resolution. Gaur, Kupper, Fischer & Hoffman. Acta Cryst. (2004). D60, 965-967
9. Replication Protein A Characterization and Crystallization of the DNA Binding Domain. Pfuetzner et al. The Journal of Biological Chemistry, Vol. 272, No. 1, Issue of January 3, pp. 430–434, 1997.

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.**

Technical Support

Please e-mail (tech@hrmail.com), fax (1-949-425-1611), or telephone (1-949-425-1321 option 2) your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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