Data Sheet

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Jena Bioscience

JBS Floppy-Choppy

In situ proteolysis as rescue technique in protein crystallization

Cat. No.	Amount
CO-110	1 Kit

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20 °C

Application

In situ proteolysis of protein samples by different proteases in the crystallization trial to enhance crystallization behavior of the protein.

Kit Contents

- 5 aliquots of 50 µl stock solutions, each of:
  - 1 mg/ml α-chymotrypsin (supplied in 1 mM HCl and 2 mM $CaCl_2$)
  - 1 mg/ml Trypsin (supplied in 1 mM HCl and 2 mM $CaCl_2$)
  - 1 mg/ml Subtilisin A (supplied in 10 mM Na acetate and 5 mM Ca acetate)
  - 1 mg/ml Papain (supplied in water)
- 4 aliquots of 1 ml buffer, containing 10 mM HEPES pH 7.5 and 500 mM NaCl

General

Analysis of structural genomics surveys show that only 15-20 % of the protein targets which can be purified will yield single crystals suitable for X-ray structure determination [1,2].

Beside the possibility to screen and optimize crystallization conditions, the protein itself can be modified to enhance the crystallization behavior. A prominent example is the reductive methylation of lysine residues on the protein surface (see JBS Methylation Kit, CO-510).

Further, it has been shown that domains or stable fragments of proteins crystallize a lot easier or yield better diffracting crystals than the intact protein. The addition of trace amounts of proteases to the protein solution immediately prior to crystallization – *in situ* proteolysis – generally results in digestion of flexible parts of the protein, i.e. trimming of the N- and/or C-termini as well as common "tags" (such as His_6). In a few instances internal loop digestion and entire domain removal has been observed [2].

Large scale application has shown that *in situ* proteolysis has doubled the success rate in protein crystallization and structure determination and therefore is one of the most efficacious crystallization rescue strategies [2].

JBS Floppy-Choppy for *in situ* proteolysis

- α-Chymotrypsin from bovine pancreas

  EC- Number: 3.4.21.1
  α-Chymotrypsin is a serine protease that selectively catalyzes the hydrolysis of peptide bonds at the carboxyl side of tyrosine, tryptophan and phenylalanine.

- Trypsin from bovine pancreas

  EC- Number: 3.4.21.4
  Trypsin belongs to the serine protease S1 family. It cleaves peptides at the carboxyl side of arginine and lysine except when either is followed by proline. Acidic residues on either side of the cleavage site lower the rate of hydrolysis.

- Subtilisin from *Bacillus licheniformis*

  EC- Number: 3.4.21.62
  Subtilisin A is grouped into the Serine S8 Endoproteinase family. It shows a broad specificity with a preference for a large uncharged residue in the P1 position. It is active under alkaline conditions.

- Papain from *papaya latex*

  EC- Number: 3.4.22.2
  Papain is a cysteine protease belonging to the peptidase C1 family. It catalyzes the hydrolysis of peptide bonds of basic amino acids, i.e. leucine and glycine. It also hydrolyzes esters and amides.

Pre-screening to identify a promising protease

Limited proteolysis can be monitored by denaturing gel electrophoresis or mass spectrometry in order to

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identify a suitable protease and its appropriate concentration yielding a promising degradation pattern. (Pre-screening is optional and may be omitted. The protein may be directly conducted to the crystallization trial after adding protease.)

Instructions for pre-screening:

1. Prepare a 1:100 dilution of an aliquot of each protease using the supplied buffer containing 10 mM HEPES pH 7.5 and 500 mM NaCl.
2. Transfer approximately 100 µg of protein (5–10 µl @ 20–10 mg/ml) into 4 vials each.
3. Add 10 µl of diluted protease (0.1 µg) per vial and incubate for thirty minutes at room temperature.
4. Quench reaction with SDS-Coomassie sample loading buffer (for gel electrophoresis) or formic acid (for mass spectrometry).
5. Analyze by SDS-PAGE or MS and select the protease which yielded the largest, most stable digestion product for subsequent crystallization experiments.
6. In case that insufficient digestion is observed repeat steps 1-5 with a 1:10 dilution of each protease.
7. In case that overdigestion is observed, repeat steps 1-5 with a 1:1,000 dilution of each protease.

In situ proteolysis

In situ proteolysis implies the addition of trace amounts of protease to the protein solution immediately prior to crystallization. Thus, crystallization experiments can be set up without evaluating the efficacy of proteolysis, without stopping the proteolysis reaction and without purification of any proteolyzed protein fragments.

However, if the protein sample is scarce, we recommend pre-screening to identify a suitable protease and concentration as described above.

Instructions for *in situ* proteolysis:

- Allow the protease stock solutions to thaw on ice.
- Add protease stock solution to the purified protein on ice immediately prior crystallization trials. Use the **amount of protease identified during pre-screening**.
- If no pre-screening has been performed, use **1 µg of protease per 100 – 10,000 µg of protein**, i.e. for a ratio of 1:100 (w/w) add 10 µl of protease stock to 100 µl of protein @ 10 mg/ml.
- Perform crystallization experiments as usual.

References

[1] Dong *et al.* (2007) In situ proteolysis for protein crystallization and structure determination. *Nature Meth.* **4**:1019.

[2] Wernimont *et al.* (2009) *In Situ* Proteolysis to Generate Crystals for Structure Determination: An Update. *PLoS ONE* **4**: e5094.