PreScission Protease (PSP)

Introduction

PreScission Protease is a recombinant protease that combines human rhinovirus type 14 3C protease and GST. This protease can specifically recognize the short peptide Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro at low temperature (4°C) and digest between Gln and Gly amino acid residues. Its substrate recognition and cleavage depend not only on the primary structure of the recombinant protease but also on its secondary structure and tertiary structure. It specifically isolates the heterologous proteins expressed by the vectors such as pGEX-6p series from its fused GST label to obtain heterologous target proteins with higher purity.

Components and Storage

Components \ Size	K1101-100U	K1101-200U	K1101-500U	Storage
PreScission Protease	100 U	200 U	500 U	-80°C
10X Cleavage Buffer	1 mL	2 x 1 mL	3 x 1 mL	-80°C
Shipping: dry ice		Shelf life: 2 years		

Note: It is recommended to aliquot upon first use to avoid repeated freeze-thaw cycles: store aliquots at -20°C for a validity period of 6 months.

Protocol

The working liquid concentration should be determined according to the specific experiment, and it is suggested that the best experimental concentration should be searched by pre-experiment.

1. Exploration of Initial Conditions

a. The enzyme digestion reaction system is set up in accordance with the following table:

Reagent	Volume
GST fusion protein	100 μg
PreScission Protease	2 μL（1U/μL）
10X Cleavage Buffer	10 μL
ddH_2O	To 100 μL

b. Place the reaction mixture at 4°C for 15-16 h;

c. Using 20 μL sample for SDS-PAGE electrophoresis analysis, according to the results, optimize the optimum amount of enzyme needed for the reaction and repeat the steps;

d. In practice, it is recommended to use 1:25-1:100 U/μg fusion protein.

2. **GST label protein digested on column** (take 10mg GST label protein/ mL gels as an example)

a. Under 4℃ conditions, the purified column was washed with 10 times the column volume enzyme digestion buffer and GST residual buffer was removed.

b. Prepared PreScission Protease: approximately 2 U PreScission Protease (per 100 labeled proteins used or in accordance with the optimized conditions after step 1). Use 200 U PreScission Protease, for 10 mg GST tag proteins to dilute to the same volume as the gel column, i.e. 1 mL., using PreScission digestion buffer.

c. The diluted 1 mL of Precision Protease was pumped into the purification column and maintained at 4°C for 4-8 h (to ensure complete enzyme digestion, enzyme digestion at 4°C for the night). If the protein binding is performed in a centrifuge tube, the prepared Precision Protease can be added directly to the centrifuge tube, shaking slowly at 4°C for 4-8 hours on a shaker (to ensure complete enzyme digestion, it can be digested overnight at 4°C).

d. The purification column was washed with PreScission Protease digestion buffer of one time the volume of the column bed, repeated three times, and each washing solution was collected separately. If the digestion reaction was carried out in a centrifuge tube, 1000g were centrifuged for 2 minutes to collect the supernatant, then 1 mL of enzyme digestion buffer was added to resuspend the precipitate. Centrifugation (1000g x 2 min) collected the supernatant and washed again. The elution component contained the target protein that removed the GST label, while the GST label and the PreScission Protease with the GST label were still bound to the gel column.

3. **GST label protein digested out of column** (10 mg GST label protein/mL gels as an example)

a. Use a desalination column to quickly remove special components such as GSH and imidazolium from the eluted fraction, or to perform dialysis with a PreScission Protease enzyme digestion buffer.

b. Add protease at a rate of 2 U PreScission Protease per 100 μg, incubate 4-8 h or stay overnight.

c. The digested protein samples were added to pre-balanced GST-labeled protein purified resin (GSTSep Glutathione Agarose Resin) with Precision Protease cutting buffer for 20-30 minutes at room temperature.

d. 500g centrifuged for 5 min to collect the supernatant containing the removed labeled target protein, whereas PreScission protease binds to the gel precipitate. If the target protein is a GST label protein, then the residual undigested GST label protein, PreScission protease, and digested GST label bind to the gel precipitate, removing the labeled target protein in solution.

Note

1. 100 mM $ZnCl_2$, 4 mM AEBSF and 100 μM Chymostatin will inhibit the enzyme activity above PreScission Protease 50%.
2. 10X Cleavage Buffer: 500mM Tris-HCl, 1.5M NaCl, 10mM DTT, pH7.5; During the experiment, the buffer can be prepared as needed according to the experimental progress.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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