PreScission Protease

Product Specification Sheet

Introduction

Product code

27084301

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

PreScission Protease is shipped on dry ice and can be stored for one week at -20°C. For longer storage it is recommended to store in small aliquots at -80°C. Avoid freeze thaw.

Description

PreScission™ Protease is a fusion protein consisting of glutathione S-transferase (GST) and human rhinovirus (HRV) type 14-3C protease (1). The protease specifically recognizes a subset of sequences which include the core amino acid sequence Leu-Phe-Gln/Gly-Pro cleaving between the Gln and Gly residues (2). Substrate recognition and cleavage are likely to be dependent not only upon primary structural signals, but also upon the secondary and tertiary structures of the fusion protein as well. Since the protease is fused to GST, it is easily removed from cleavage reactions using Glutathione Sepharose™ 4B. Fusion proteins produced from pGEX-6P-1 (28-9546-48), pGEX-6P-2 (28-9546-50) and pGEX-6P-3 (28-9546-51) will be cleaved by PreScission Protease between the GST moiety and the cloned fusion partner. The molecular weight of PreScission Protease is approximately 46 kDa.

Protocol overview

Purification of GST-tagged protein using Glutathione Sepharose 4B, Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose High Perfomance

Clarified bacterial lysate containing the expressed recombinant, GST-tagged protein is loaded onto a column or 96-well filter plate packed with Glutathione Sepharose. The column is then washed with binding buffer to remove non-specifically bound proteins and the recombinant GST-tagged protein is eluted in a buffer containing 10 mM glutathione (reduced). Alternatively, the sample can be batch purified directly in a tube or beaker. All components required for purification by Glutathione Sepharose formats utilizing Glutathione Sepharose 4B, Glutathione 4 Fast Flow and Glutathione Sepharose High Performance affinity media.

Cleavage Protocols

During cleavage reactions, it is recommended that samples be removed at various time points and analyzed by SDS-PAGE to estimate the yield, purity and extent of digestion. The amount of PreScission Protease, temperature and length of incubation required for complete digestion of a given GST fusion protein may vary depending on the fusion partner. Optimal conditions for each fusion should be determined in pilot experiments.

PreScission Protease can be used to digest GST-tagged proteins using one of two methods:

On-column cleavage of fusion proteins

It is recommended that PreScission Protease be added to Glutathione Sepharose to which a GST fusion protein has been bound. This method allows simultaneous cleavage of the GST fusion protein and removal of PreScission protease from the sample. The flow-through will contain the fusion partner which has been cleaved from the GST moiety. Both PreScission Protease and the GST moiety will be retained by the Glutathione Sepharose. If residual PreScission Protease remains in the flow-through, it can be removed by passing the sample over fresh Glutathione Sepharose.

Buffer preparations

Prepare cleavage buffer before digestion and chill to 5°C prior to use:

Note: *Digestion may be improved by adding Triton™ X-100, Tween™ 20, Nonidet or NP40 to a concentration of 0.01% Concentrations of these detergents up to 1% do not inhibit PreScission Protease*

Cleavage buffer: 50 mM Tris-HCl; pH 7.0 (at 25°C); 150 mM NaCl; 1 mM EDTA; 1 mM dithiothreitol.

Step	Action
1	Bind the GST fusion protein sonicate to the appropriate bed volume[1] of washed and equilibrated Glutathione Sepharose at 5°C. Detailed instructions for the purification of GST fusion proteins are provided in the GST Purification Modules (27-4570-01, -02). Specific instructions may also be found in the GST Gene Fusion Manual, available from your Cytiva representative.
2	Wash the fusion protein-bound matrix with 10 bed volumes of Cleavage Buffer at 5°C and remove residual buffer.
3	For each mL of washed Glutathione Sepharose bed volume, mix 40 µL (80 units) of PreScission Protease with 960 µL of Cleavage Buffer at 5°C.
4	Add the PreScission Protease mixture to the fusion protein-bound Glutathione Sepharose and gently resuspend.
5	Incubate at 5°C for 4 hours. ***Note:*** *More rapid cleavage may be achieved by adding a greater amount of PreScission Protease.*

[1] Bed volume is equal to 0.5X the volume of a 50% Glutathione Sepharose slurry used or 0.75X the volume of the original Glutathione Sepharose slurry supplied in the Bulk GST Purification Module. RediPack columns contain a 2 mL bed volume.

Step	Action
6	Collect eluate by either centrifugation of bulk Glutathione Sepharose matrix at 500 × g for 5 minutes or by collecting the eluate as it flows from the Glutathione Sepharose column. The eluate will contain the protein of interest, while the GST portion of the fusion protein and the PreScission Protease remain bound to the Glutathione Sepharose matrix.

Cleavage in solution

Buffer preparations

Prepare cleavage buffer before digestion and chill to 5°C prior to use:

Note: *Digestion may be improved by adding Triton X-100, Tween 20, Nonidet or NP40 to a concentration of 0.01% Concentrations of these detergents up to 1% do not inhibit PreScission Protease.*

Cleavage buffer: 50 mM Tris-HCl; pH 7.0 (at 25°C); 150 mM NaCl; 1 mM EDTA; 1 mM dithiothreitol.

Step	Action
1	Following elution of the GST fusion protein from Glutathione Sepharose, dialyze the eluate extensively against Cleavage Buffer in order to remove reduced glutathione from the sample. ***Note:*** *Alternatively, sample may be adjusted to 1X Cleavage Buffer by addition of the appropriate volume of 10X Cleavage Buffer.*
2	Add 1 µL (2 units) of PreScission Protease for each 100 µg of fusion protein in the eluate. If the amount of fusion protein in the eluate has not been determined, add 40 µL (80 units) of PreScission Protease for each ml of Glutathione Sepharose bed volume[1] from which the fusion protein was eluted.
3	Incubate at 5°C for 4 hours. ***Note:*** *More rapid cleavage may be achieved by adding a greater amount of PreScission Protease. Glutathione Sepharose slurry supplied in the Bulk GST Purification Module.*

[1] Bed volume is equal to 0.5X the volume of a 50% Glutathione Sepharose slurry used or 0.75X the volume of the original

Step	Action
4	Once digestion is complete, apply the sample to washed and equilibrated Glutathione Sepharose to remove the GST portion of the fusion protein and the PreScission Protease from the protein of interest. Detailed instructions for the purification of GST fusion proteins are provided in the GST Purification Module (27-4570-01). Specific instructions may also be found in the GST Gene Fusion Manual, available from your Cytiva representative.

Troubleshooting

PreScission Protease cleavage is incomplete

- *PreScission Protease to fusion protein ratio is incorrect:* Check the amount of fusion protein in the digestion. Adjust the amount of PreScission Protease added to at least 10 units/mg of fusion protein. If performing on-column cleavage, note that the capacity of Glutathione Sepharose for GST is typically ≥8 mg/ml. In most purifications, however, the resin is not saturated with fusion protein.
- *Increase incubation time and enzyme concentration:* If the fusion protein is not degraded by extensive incubation with PreScission Protease, increase the reaction time to 20 hours and increase the amount of PreScission Protease used in the reaction.
- *Verify presence of PreScission Protease cleavage site:* Compare the DNA sequence of the construct with the known PreScission Protease cleavage sequence. Verify that the optimal PreScission Protease recognition site, Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro, has not been altered during the course of cloning your fusion protein.
- *Ensure absence of PreScission Protease inhibitors:* Dialyze the fusion protein against Cleavage Buffer before cleaving with PreScission Protease. The presence of Zn2+ as well as other inhibitors (see *Reference information, on page 6*) will interfere with the activity of PreScission Protease.

Multiple bands are observed on SDS Gel following PreScission Protease cleavage

- *Determine when bands appear:* Test to be certain that additional bands are not present prior to PreScission Protease cleavage. These may be the result of proteolysis in the host bacteria. If this is the case, the use of a protease-deficient strain (e.g., lon- or ompT) such as *E. coli* BL21 may be required. *E. coli* BL21 is provided with each pGEX-6P vector.

- *Fusion partner may contain recognition sequences for PreScission Protease:* PreScission Protease optimally recognizes the sequence Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro and cleaves between the Gln and Gly residues, however other similar secondary sites may exhibit some propensity for cleavage. Check the sequence of the fusion partner (if possible) for the presence of additional PreScission Protease recognition sites. Adjusting reaction conditions (e.g., time, temperature and salt concentration) may result in selective cleavage at the desired site. If adjustment of the conditions does not correct the problem, reclone the insert into a pGEX expression vector encoding the recognition sequence for either thrombin or factor Xa proteases.

The fusion partner is contaminated with PreScission Protease after purification with Glutathione Sepharose

- *Pass the sample over fresh Glutathione Sepharose:* If the Glutathione Sepharose was saturated with GST fusion protein, not all of the PreScission Protease may have been removed by the matrix. Use of a second round of Glutathione Sepharose chromatography should remove the residual PreScission Protease.

Reference information

- For more information on the use of pGEX vectors, request the GST Gene Fusion System Manual from your local Cytiva representative.
- General PreScission Protease cleavage conditions (PreScission Protease at ≥10 units/mg GST fusion protein)

pH	7 - 8
Temperature	5 - 15°C
Dithiothreitol	1 mM
NaCl	0 - 500 mM
EDTA	0 - 10 mM
Detergents (Triton X-100, Tween-20, NP-40, Nonidet)	0 - 1% (v/v)

- The following reagents inhibit PreScission Protease cleavage activity by more than 50% at the indicated concentrations:

$ZnCl_2$	100 mM
Pefabloc™ 7 SC	4 mM
Chymostatin	100 µM

- The following reagents do not inhibit PreScission Protease activity by more than 20% at the indicated concentrations:

Antipain dihydrocloride	74 µM
Aprotinin	0.3 µM
Bestatin	130 µM
E-64	28 µM
Leupeptin	1 µM
Pepstatin	1 µM
Phosphoramidon	0.6 mM
PMSF	1 mM
$ZnCl_2$	10 mM

- This product does not contain, and is not derived from, specified risk materials as defined in Commission Decision 97/534/EC.

References

1. Walker, P.A. *et al., BIO/TECHNOLOGY* 12, 601 (1994).
2. Cordingly, M.G. *et al., J. Biol. Chem*. 265, 9062 (1990).

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