

# **ab207000**

## **Thrombin cleavage kit**

Instructions for use:

For efficiently removing tags from recombinant fusion proteins containing an accessible thrombin cleavage sequence.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

---

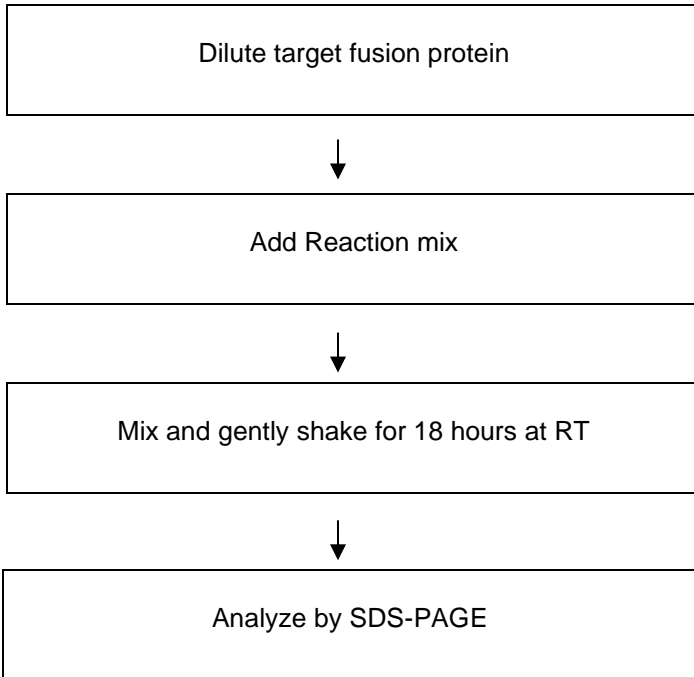
<b>INTRODUCTION</b>	1
<b>1. BACKGROUND</b>	1
<b>2. ASSAY SUMMARY</b>	2
<b>GENERAL INFORMATION</b>	3
<b>3. PRECAUTIONS</b>	3
<b>4. STORAGE AND STABILITY</b>	3
<b>5. LIMITATIONS</b>	3
<b>6. MATERIALS SUPPLIED</b>	4
<b>7. MATERIALS REQUIRED, NOT SUPPLIED</b>	4
<b>8. TECHNICAL HINTS</b>	4
<b>ASSAY PREPARATION</b>	5
<b>9. REAGENT PREPARATION</b>	5
<b>ASSAY PROCEDURE</b>	6
<b>10. ASSAY PROCEDURE</b>	6
<b>DATA ANALYSIS</b>	8
<b>11. TYPICAL DATA</b>	8
<b>RESOURCES</b>	10
<b>12. QUICK ASSAY PROCEDURE</b>	10
<b>13. NOTES</b>	11

## 1. BACKGROUND

Abcam's Thrombin cleavage kit (ab207000) efficiently removes tags from recombinant fusion proteins containing an accessible thrombin cleavage sequence.

Thrombin is a valuable biochemical tool due to its high proteolytic specificity. A thrombin cleavage site (e.g., Leu-Val-Pro-Arg-II-Gly-Ser; where II denotes the cleavage site) is widely incorporated within the linker region of fusion or affinity tagged recombinant proteins. After successful cleavage with thrombin, affinity tags or fused proteins can be separated from the target protein. Abcam's Thrombin cleavage kit provides an easy approach to test and optimize cleavage conditions of a target fusion or affinity-tagged protein containing a thrombin-specific cleavage site. The kit contains active thrombin enzyme sufficient to cleave up to 5 mg of the target protein. A 6x His-tagged protein containing the thrombin cleavage site is included as a cleavage control protein. Following cleavage of the target protein, thrombin can be removed by passing the reaction mix through a Heparin Sepharose® column.

## 2. ASSAY SUMMARY



### 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

### 4. STORAGE AND STABILITY

**Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 6 and 9.

### 5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## GENERAL INFORMATION

### 6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Thrombin Dilution Buffer	1 mL	-20°C	-20°C
Thrombin Cleavage Buffer	25 mL	-20°C	-20°C
Active Thrombin	10 µL	-20°C	-80°C
Thrombin Cleavage Control	1 vial	-20°C	-20°C

### 7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Sterile microcentrifuge tubes or disposable 15 mL or 50 mL tubes

### 8. TECHNICAL HINTS

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.

## 9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

### 9.1. **Thrombin Dilution Buffer:**

Ready to use. Bring to room temperature before use.

### 9.2. **Thrombin Cleavage Buffer:**

Ready to use. Bring to room temperature before use.

### 9.3. **Active Thrombin:**

Prepare a stock solution of Active Thrombin (1 U/ $\mu$ L) by adding 550  $\mu$ L of the Thrombin Dilution Buffer to the Active Thrombin. Mix well by pipetting up and down (do not vortex). Aliquot and store at  $-80^{\circ}\text{C}$ . Avoid repeated freeze/thaw.

### 9.4. **Thrombin Cleavage Control:**

Reconstitute with 40  $\mu$ L of deionized water to obtain 1 mg/mL Thrombin Cleavage Control solution. Once reconstituted, aliquot and store at  $-20^{\circ}\text{C}$  for up to 6 months. Avoid repeated freeze/thaw.

## ASSAY PROCEDURE

### 10. ASSAY PROCEDURE

- 10.1. Dilute your target fusion protein to a final concentration of 1 mg/mL with appropriate volume of Thrombin Cleavage Buffer.
- 10.2. Use 1 sterile microcentrifuge tube per cleavage reaction. Add the following reagents to each tube. Include 1 cleavage reaction with Thrombin Cleavage Control.

Component	Target Protein Mix (10 µg)	Thrombin Cleave Control/Cleavage Control Protein Mix (10 µg)
Control Protein	-	10 µL
Target Protein	10 µL	-
Thrombin Cleavage Buffer	39 µL	39 µL
Active Thrombin (1 U/µL)	1 µL	1 µL

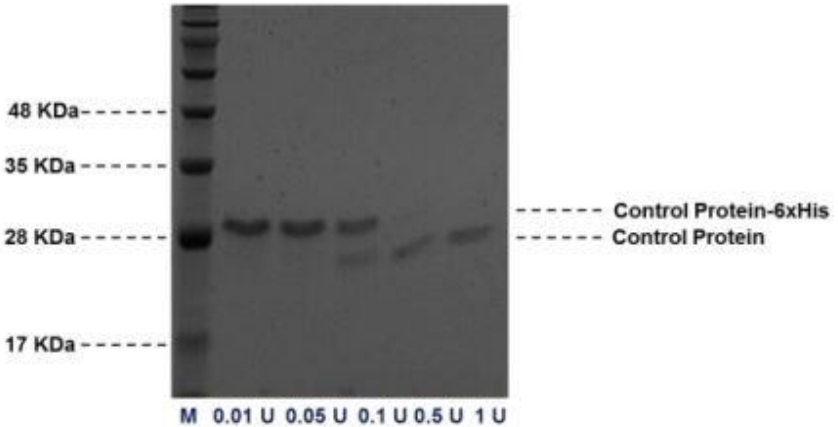
- 10.3. Mix gently by pipetting up and down (do not vortex).
- 10.4. Gently shake at room temperature for 18 hours.
- 10.5. Take out 10 µL from the target protein reaction mixtures at intervals of 0, 2, 4, 6 and 18 hours after setting up the reaction mixture and freeze at -20°C.
- 10.6. After 18 hours, analyze all the time point samples removed from each reaction by SDS-PAGE, along with 2-3 µL of undigested Thrombin Cleavage Control.

**Note:** In order to find the optimum cleavage conditions, it is recommended to run preliminary cleavage reactions on a small scale. The enzyme stock solution (1 U/µL) maybe further diluted with the Thrombin Cleavage Buffer to obtain enzyme solutions containing 0.01, 0.05, 0.1 and 0.5 U of thrombin. Once optimum cleavage conditions are obtained, the reaction can be scaled up to cleave the entire amount of the target protein. Successful cleavage with thrombin is dependent upon proper folding and lack of aggregation of the fusion protein to enable the enzyme to access the thrombin recognition sequence.

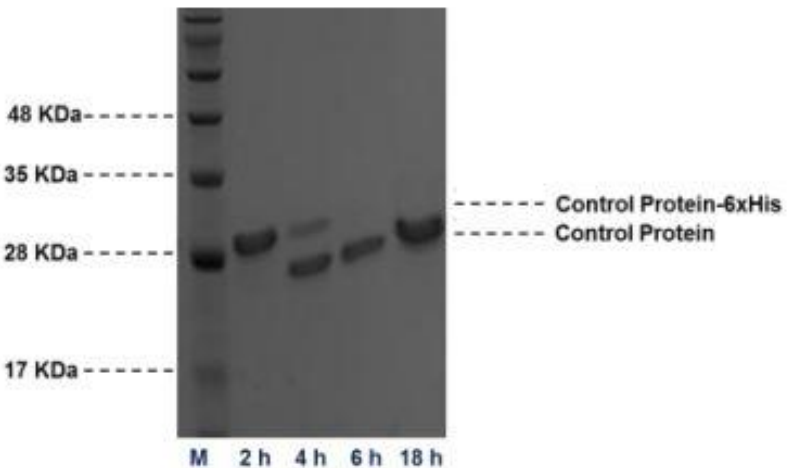
## ASSAY PROCEDURE

*One unit of Thrombin is the amount of enzyme required to cleave 10  $\mu$ g of the provided Thrombin Cleavage Control to 95% completion when incubated in the Thrombin Cleavage Buffer at 20°C for 18 hours.*

## 11. TYPICAL DATA



**Figure 1:** SDS-PAGE analysis of thrombin cleavage using different amount of thrombin and ab207000: Cleavage of 10  $\mu$ g of 6x His-tagged Thrombin Cleavage Control with different amounts (0.01-1 U/ $\mu$ L) of thrombin at room temperature for 18 hours.



**Figure 2:** SDS-PAGE analysis of thrombin cleavage at different time points using ab207000: Cleavage of 10  $\mu$ g of 6x His-tagged Thrombin Cleavage Control.

## RESOURCES

### 12. QUICK ASSAY PROCEDURE

**NOTE:** This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Dilute target fusion protein to a final concentration of 1 mg/mL with appropriate volume of Thrombin Cleavage Buffer.
- Prepare Reaction Mix:

Component	Target Protein Mix (10 µg)	Thrombin Cleavage Control Mix (10 µg)
Control Protein	N/A	10 µL
Target Protein	10 µL	N/A
Thrombin Cleavage Buffer	39 µL	39 µL
Active Thrombin (1 U/µL)	1 µL	1 µL

- Agitate at room temperature for 18 hours.
- Analyze all time point samples by SDS-PAGE along with 2-3 µL of undigested Thrombin Cleavage Control.

## 13. NOTES

## RESOURCES

## RESOURCES

## **Technical Support**

Copyright © 2025 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)