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.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6a, Last Updated 16 August 2023

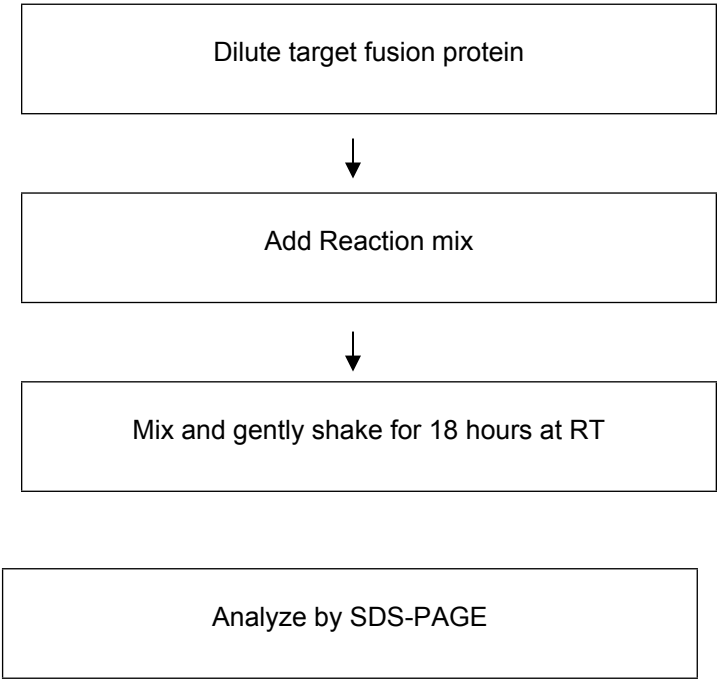
Table of Contents

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-Il-Gly-Ser; where Il 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.

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/Thrombin Enzyme	1 vial	-20°C	-80°C
Thrombin Cleavage Control/Cleavage Control Protein (Lyophilized)	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/Thrombin Enzyme:**

Prepare a stock solution of Active Thrombin/Thrombin Enzyme (1 U/µL) by adding 550 µL of the Thrombin Dilution Buffer to the Active Thrombin/Thrombin Enzyme. Mix well by pipetting up and down (do not vortex). Aliquot and store at -80°C. Avoid repeated freeze/thaw.

9.4. **Thrombin Cleavage Control/Cleavage Control Protein:**

Reconstitute with 40 µL of deionized water to obtain 1 mg/mL Thrombin Cleavage Control/Cleavage Control Protein solution. Once reconstituted, aliquot and store at -20°C for up to 6 months. Avoid repeated freeze/thaw.

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/Cleavage Control Protein.

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/Thrombin Enzyme (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/Cleavage Control Protein.

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 µg of the provided Thrombin Cleavage Control/cleavage control protein to 95% completion when incubated in the Thrombin Cleavage Buffer at 20°C for 18 hours.

11. TYPICAL DATA

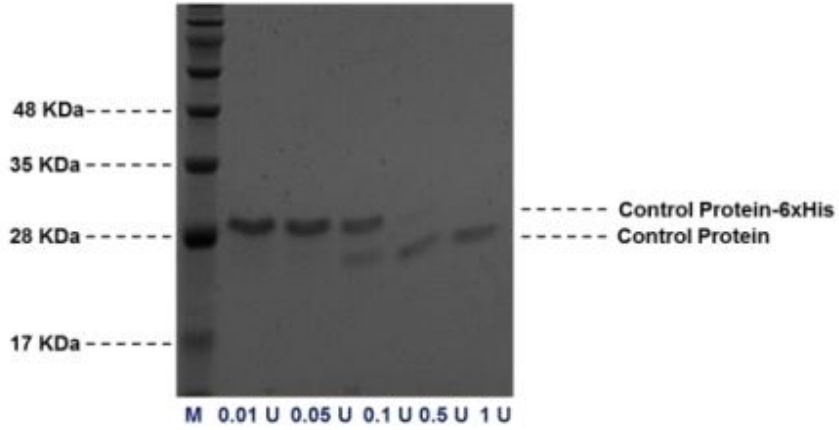


Figure 1: SDS-PAGE analysis of thrombin cleavage using different amount of thrombin and ab207000: Cleavage of 10 µg of 6x His-tagged Thrombin Cleavage Control/Cleavage Control Protein with different amounts (0.01-1 U/µL) of thrombin at room temperature for 18 hours.

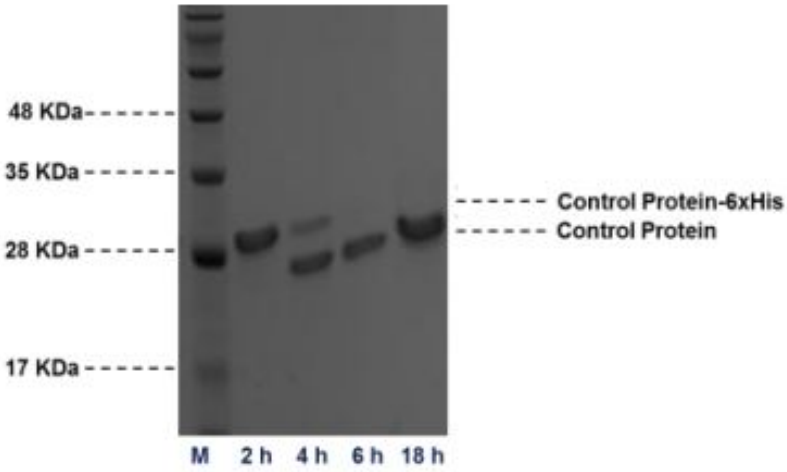


Figure 2: SDS-PAGE analysis of thrombin cleavage at different time points using ab207000: Cleavage of 10 µg of 6x His-tagged Thrombin Cleavage Control/Cleavage Control Protein.

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/Cleavage Control Protein Mix (10 µg)
Control Protein	N/A	10 µL
Target Protein	10 µL	N/A
Thrombin Cleavage Buffer	39 µL	39 µL
Active Thrombin/Thrombin Enzyme (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/Cleavage Control Protein.

13. NOTES

RESOURCES

RESOURCES

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)