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.

Version 4 Last Updated 18 December 2015
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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-Ile-Gly-Ser; where Ile 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.
INTRODUCTION

2. ASSAY SUMMARY

Dilute target fusion protein

Add Reaction mix

Mix and gently shake for 18 hours at RT

Analyze by SDS-PAGE
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

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin Dilution Buffer</td>
<td>250 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Thrombin Cleavage Buffer</td>
<td>20 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Thrombin Enzyme</td>
<td>10 x 1 mL</td>
<td>-20°C</td>
<td>-80°C</td>
</tr>
<tr>
<td>Cleavage Control Protein (Lyophilized)</td>
<td>20 x 1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

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

9.4. Cleavage Control Protein:
Reconstitute with 40 μL of deionized water to obtain 1 mg/mL 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 Cleavage Control Protein.

<table>
<thead>
<tr>
<th>Component</th>
<th>Target Protein Mix (10 µg)</th>
<th>Cleavage Control Protein Mix (10 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Protein</td>
<td>-</td>
<td>10 µL</td>
</tr>
<tr>
<td>Target Protein</td>
<td>10 µL</td>
<td>-</td>
</tr>
<tr>
<td>Thrombin Cleavage Buffer</td>
<td>39 µL</td>
<td>39 µL</td>
</tr>
<tr>
<td>Thrombin Enzyme (1 U/µL)</td>
<td>1 µL</td>
<td>1 µL</td>
</tr>
</tbody>
</table>

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 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.

One unit of Thrombin is the amount of enzyme required to cleave 10 µg of the provided 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 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 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Target Protein Mix (10 µg)</th>
<th>Cleavage Control Protein Mix (10 µg)</th>
</tr>
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<tbody>
<tr>
<td>Control Protein</td>
<td>N/A</td>
<td>10 µL</td>
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<tr>
<td>Target Protein</td>
<td>10 µL</td>
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<td>Thrombin Cleavage Buffer</td>
<td>39 µL</td>
<td>39 µL</td>
</tr>
<tr>
<td>Thrombin Enzyme (1 U/µL)</td>
<td>1 µL</td>
<td>1 µL</td>
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- Agitate at room temperature for 18 hours.
- Analyze all time point samples by SDS-PAGE along with 2-3 µL of undigested Cleavage Control Protein.
13. NOTES
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