FOR RESEARCH USE ONLY!

Thrombin Cleavage Kit

rev 09/20

(Catalog # K377-500; 500 reactions, Store kit at -20°C)

I. Introduction:

Thrombin enzyme (Factor IIa, EC 3.4.21.5) is an important clotting factor that controls the transformation of soluble fibrinogen to insoluble active fibrin strands. It is also used as a valuable biochemical tool due to its high proteolytic specificity. Thrombin cleavage site (e.g. Leu-Val-Pro-Arg-Il-Gly-Ser; Il: 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. **Biovision's Thrombin Cleavage Kit** provides an easy approach to test and optimize cleavage conditions of a target fusion or affinity-tagged protein containing Thrombin-specific cleavage site. The kit contains an active Thrombin enzyme sufficient to cleave up to 5 mg of the target protein. A 6xHis-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 the Heparin Sepharose column (BioVision Cat # 6553 and 6554).

II. Application:

Efficiently remove tags from recombinant fusion proteins containing accessible Thrombin-specific recognition sequence.

III. Kit Contents:

Components	K377-500	Cap Code	Part Number
Thrombin Dilution Buffer	1 ml	Clear	K377-500-1
Thrombin Cleavage Buffer	25 ml	WM	K377-500-2
Thrombin Enzyme	1 vial	Green	K377-500-3
Cleavage Control Protein	Lyophilized	Red	K377-500-4

IV. User Supplied Reagents and Equipment:

- Sterile Eppendorf tubes or Falcon tubes.

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Briefly centrifuge all small vials at low speed prior to opening. Read the entire protocol before performing the assay.

- **Thrombin Dilution and Cleavage Buffer:** Bring to room temperature (RT) before use.
- **Thrombin Enzyme:** Prepare a stock solution of the Thrombin Enzyme by adding 550 µl of the Thrombin Dilution Buffer to the Thrombin Enzyme. Mix well by pipetting up and down (Do not vortex). Divide into aliquots & store at -80°C. Avoid repeated freeze/thaw.
- **Cleavage Control Protein:** Reconstitute with 40 µl of deionized water to obtain 1 mg/ml Cleavage Control Protein solution.

VI. Thrombin Cleavage Protocol:

a. Target Protein: Dilute your target fusion protein to final concentration of 1mg/ml with appropriate volume of Thrombin Cleavage Buffer.

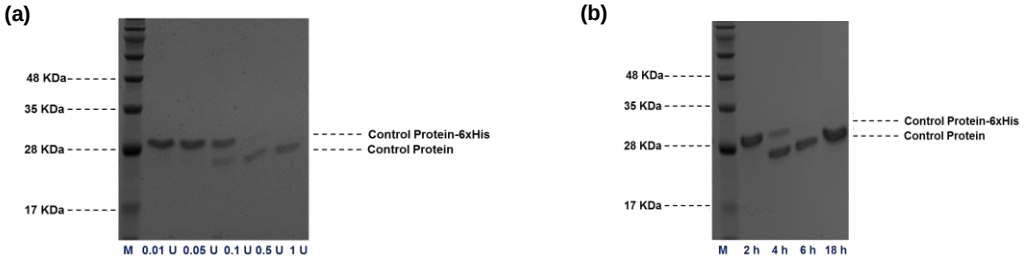
b. Reaction Mix: In a sterile Eppendorf tube, mix enough of the following reagents for the number of cleavage reactions to be performed:

	Target Protein Mix (10 µg)	Cleavage Control Protein Mix (10 µg)
Control Protein	-	10 µl
Target Protein	10 µl	-
Thrombin Cleavage Buffer	39 µl	39 µl
Thrombin Enzyme (1 U/µl)	1 µl	1 µl

Mix gently by pipetting up and down (do not vortex) and gently shake at room temperature (RT) for 18 hrs. Take out ~10 µl from the target protein reaction mixture at the intervals of 2, 4, 6 and 18 hrs and freeze at -20°C. After 18 hrs, analyze all time reaction variants 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 at a small scale. For that, the enzyme stock solution (1 U/µl) can be further diluted with the Thrombin Cleavage Buffer to obtain an enzyme solution containing 0.01, 0.05, 0.1 and 0.5 U. Successful cleavage with Thrombin is dependent upon proper folding of the fusion protein that enables access of the Thrombin recognition sequence by the enzyme. Once optimum cleavage conditions are obtained, the reaction can be scaled up to cleave the entire amount of the target protein.

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 hrs.



Figures: SDS-PAGE analysis of Cleavage Control Protein: (a) Cleavage of 10 µg of 6xHis-tagged cleavage control protein with different amounts (0.01-1 U/ µl) of Thrombin at room temperature for 18 hrs. (b) Cleavage of 10 µg of 6xHis-tagged cleavage control protein at different time intervals.

VII. RELATED PRODUCTS:

Thrombin, Active, Bovine Plasma (7592)
Antithrombin III (7298)
Thrombin Activity Fluorometric Assay Kit (K373)
Thrombin Inhibitor Screening Kit (Fluorometric) (K374-100)
Heparin Sepharose Column (6554)
Thrombin, Active, Human Plasma (7593)
Factor Xa Inhibitor Screening Kit (Fluorometric) (K362-100)
Factor Xa Activity Fluorometric Assay kit (K361)
Heparin-Sepharose (6553)
PPACK dihydrochloride (1848)

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