MESGEN
INNOVATION BIOTECHNOLOGY

Thrombin protease

(lyophilized powder, Cat#ZJ9001)

Background

Thrombin enzyme (Activated Factor IIa) is an important clotting promoter that controls the transformation of soluble fibrinogen to insoluble active fibrin strands. Thrombin is a coagulation protein and a serine protease (EC 3.4.21.5) that catalyzes many coagulation-related reactions. Thrombin triggers factor-XI, factor-V, Factor-XIII and factor-VIII. Thrombin endorses platelet activation, using activation of protease-activated receptors on the platelet. As a result of its high proteolytic specificity, thrombin has become an important biochemical protein. The thrombin cleavage site (Leu-Val-Pro-Arg-Gly-Ser) is widely used in linker regions of recombinant fusion protein constructs. After the purification of the fusion protein, thrombin is used to cleave between the Arginine and Glycine residues of the cleavage site, efficiently removing the purification tag from the protein of interest with a high degree of specificity.

Size and Purity(%)

1000 U per package, >97%

Reagents required

Thrombin : *MeGen (Cat#ZJ9001). Dissolve 1000 cleavage units in 1.0ml cold PBS. Swirl gently to dissolve. To preserve activity keeps the enzyme in aliquots at -80°C. One unit cleaves theoretical 100ug protein in 16 hr at 22°C in buffer.*

Note : *The amount of thrombin, temperature and length of incubation must be calibrated for each system. Samples must be removed at various time points and analyzed by PAGE-SDS to estimate the yield, purity and extent of thrombin digestion. Excess thrombin may result in unwanted proteolysis at secondary sites. Avoid the presence of serine-protease inhibitors (like PMSF or AEBSF) during reaction. Theoretically, cleavage must be complete following ON treatment with <10 cleavege unit per mg protein. For some applications thrombin must be subsequently removed from the sample by chromatography or affinity with pAminoBenzamidine - Agarose.*

1 X PBS buffer: *140mM NaCl; 2.7mM KCL; 10mM Na_2HPO_4; 1.8mM KH_2PO_4; pH 7.3*

Thrombin Cleavage of Free Eluted Fusion protein

First you have to calibrate amount of thrombin, temperature and length of incubation, taking in mind that one unit cleaves theoretical 100ug protein in 16hr at 22°C in buffer. You can incubate enough eluate to see on PAGE-SDS gels (~3ug) with 0.01, 0.03 & 0.06 Units of thrombin 2hr, 4hr, 6hr & ON

at 22°C (or RT). Stop the reaction with PAGE-SDS sample buffer + 1mM PMSF and keep immediately at -20°C until use. Analyze PAGE-SDS gels versus a control of non-cleavaged protein. Longer incubation, more enzyme or higher temperature will increase cleavage; while lower incubation, less enzyme or lower temperature will decrease cleavage.

As a general protocol you can use:

1. To the eluate from either batch or column purification, add 10ul of thrombin solution (10 cleavage units) per mg fusion protein.

2. Mix gently and incubate at RT for 2-16hrs.

3. Once digestion is complete you can stop protease cleavage with 1mM PMSF or AEBSF (more stable) and check results by PAGE-SDS gels or immediately separate the cleavage products by chromatography.

4. When a satisfactory condition is found, scale-up the reaction proportionally.

Thrombin Cleavage of Fusion protein Bound to column matrix

First you have to calibrate the amount of thrombin, temperature and length of incubation, taking in mind that one unit cleaves theoretical 100ug protein in 16hr at 22°C in buffer or one unit cleaves theoretical 20ul of bed volume saturated resin in 16hr at 22°C in buffer. You can incubate 20ul of bed volume saturated resin with 0.1, 0.5, 1 & 2 Units of thrombin (in 20ul total PBS) 2hr, 4hr, 6hr & ON at 22°C (or RT). Stop reaction by spinning for 4min 3000rpm, separate supernatant from beads, and add PAGE-SDS sample buffer + 1mM PMSF to the supernatant, and keep immediately at -20°C until use. Analyze PAGE-SDS gels versus a control of non-cleavaged protein (eluted non cleaved protein). Longer incubation, more enzyme or higher temperature will increase cleavage; while lower incubation, less enzyme or lower temperature will decrease cleavage

As a general protocol you can use:

1. For 1ml of bed volume saturated resin, mix 50Units of thrombin solution in 1ml PBS. Gently swirl to mix. Shake or rotate at 22°C (or RT) 2-16hrs.

2. Spin the suspension 4min 3000rpm to pellet the beads. Keep supernatant aside. You can stop protease cleavage with 1mM PMSF or AEBSF (more stable).

3. Extract beads twice or more with 1ml PBS or buffer. Keep each supernatant aside.

4. As a control you can elute the remaining uncleaved protein (still attached to the resin through the GST) by extraction several times with elution buffer.

5. Check results by PAGE-SDS gels or immediately separate the cleavage products by chromatography.

6. When a satisfactory condition is found, scale-up the reaction proportionally.

Stability

Store at 4°C if entire vial will be used within 7 days. Store, frozen at -20°C for longer periods of time. For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA). Avoid multiple freeze-thaw cycles.