

Thrombin cleavage assay

The amount of each material were listed in the Table 1.

All the chemicals used in the protocol were listed in the Table 2.

Thrombin cleavage assay protocol was extracted from **Novagen**.

1. Make the dilution of thrombin in the Thrombin dilution buffer. The dilution will contain approximately 0.04 U enzyme per μL .
2. Assemble the following components in a tube. Incubate the reactions at room temperature, taking 10 μL aliquots into 10 μL 2X SDS sample buffer every 15 minutes within 16 hours.
3. Determine the extent of cleavage of the samples by SDS-PAGE analysis.

Table 1. The amount of each material in the protocol.

Name	Volume
10X Thrombin Cleavage/Capture Buffer	5 μL
Target protein	10 μg
Diluted thrombin	1 μL
deionized water	X μL
Total volume	50 μL

Table 2. The information of the used chemicals in the protocol.

Name	CAS number	Brand
Thrombin	9002-04-4	Novagen