



# Human Thrombin ELISA Kit

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**Hinweis/Note:**

Der Packungsbeileger dient nur als erste Information.  
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.  
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at [support@assaypro.com](mailto:support@assaypro.com).

Thank you for choosing Assaypro.

## Assay Summary

Add 50  $\mu$ l of Standard/ Sample per well.  
Incubate 2 hours.



Wash, then add 50  $\mu$ l of  
Biotinylated Antibody per well.  
Incubate 1 hour.



Wash, then add 50  $\mu$ l of  
SP Conjugate per well.  
Incubate 30 minutes.



Wash, then add 50  $\mu$ l of  
Chromogen Substrate per well.  
Incubate 10 minutes.



Add 50  $\mu$ l of Stop Solution per well.  
Read at 450 nm immediately.





# AssayMax Human Thrombin ELISA Kit

Catalog No. ET4010-1  
Sample Insert/Reference Only

## Introduction

Thrombin (activated Factor II [IIa]) is a coagulation protein that has many effects in the coagulation cascade. Thrombin is a serine protease (EC 3.4.21.5) that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions (1). Thrombin is in the form of  $\alpha$ -thrombin that is the immediate end product of prothrombin activation: two further thrombin products can be identified,  $\beta$ - and  $\gamma$ -thrombin. These are degraded forms that may arise from autodigestion of a thrombin preparation (2, 3).

## Principle of the Assay

The AssayMax Human Thrombin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of  $\alpha$ -thrombin in human cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures thrombin in less than 4 hours. A monoclonal antibody specific for thrombin has been pre-coated onto a 96-well microplate with removable strips. Thrombin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for thrombin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

## Reagents

- **Human Thrombin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against  $\alpha$ -thrombin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Thrombin Standard:** Purified human thrombin in a buffered protein base (30 ng, lyophilized).
- **Biotinylated Human Thrombin Antibody (80x):** An 80-fold biotinylated polyclonal antibody against thrombin (100  $\mu$ l).
- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80  $\mu$ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

## Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at  $-20^{\circ}\text{C}$ .
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at  $2-8^{\circ}\text{C}$ .
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at  $2-8^{\circ}\text{C}$ .
- Store Standard at  $2-8^{\circ}\text{C}$  before reconstituting with diluent and at  $-20^{\circ}\text{C}$  after reconstituting with diluent.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20  $\mu$ l, 20-200  $\mu$ l, 200-1000  $\mu$ l, and multiple channel).
- Deionized or distilled reagent grade water.

## Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants, dilute if necessary

and assay. The undiluted samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

## Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 30 ng of Human Thrombin Standard with 1.5 ml of EIA Diluent to generate a 20 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the thrombin standard solution (20 ng/ml) 1:2 with equal volume of EIA Diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Thrombin] (ng/ml)
P1	Standard (20 ng/ml)	20.00
P2	1 part P1 + 1 part EIA Diluent	10.00
P3	1 part P2 + 1 part EIA Diluent	5.000
P4	1 part P3 + 1 part EIA Diluent	2.500
P5	1 part P4 + 1 part EIA Diluent	1.250
P6	1 part P5 + 1 part EIA Diluent	0.625
P7	1 part P6 + 1 part EIA Diluent	0.313
P8	EIA Diluent	0.000

- **Biotinylated Human Thrombin Antibody (80x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

## Assay Procedure

- Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50  $\mu$ l of Human Thrombin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200  $\mu$ l of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300  $\mu$ l of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50  $\mu$ l of Biotinylated Human Thrombin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50  $\mu$ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50  $\mu$ l of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50  $\mu$ l of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

## Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.  
To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm

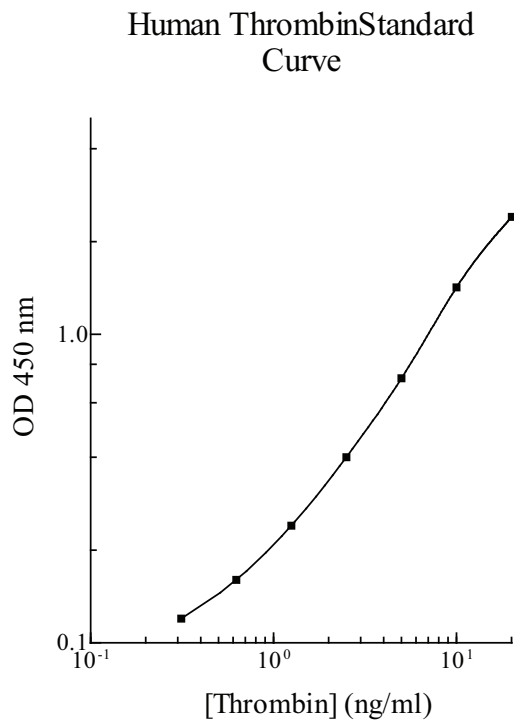


absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

### Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



### Performance Characteristics

- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.2% respectively.
- The minimum detectable dose of thrombin is typically ~ 0.3 ng/ml.
- This assay recognizes both natural and recombinant human thrombin.

### Recovery

<b>Standard Added Value</b>	0.625 – 10 ng/ml
<b>Recovery %</b>	87 – 109%
<b>Average Recovery %</b>	98%

## Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	<20%
Mouse	None
Rat	None
Swine	None
Protein	% Cross Reactivity
Prothrombin	<70%

## References

- (1) Badimon L *et al.* (1988) *Circulation* 78:1431-1442
- (2) Esmon C T *et al.* (1974) *Journal of Biological chemistry* 249: 7798-7807
- (3) Hatton M W C *et al.* (1978) *Thrombosis Research* 13: 655-670

Version 4.5

## Related Products

- EP3022-1 AssayMax Human Prothrombin ELISA Kit (Plasma, Milk, Urine, and Cell Culture samples)
- EMP3022-1 AssayMax Mouse Prothrombin ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)
- EPP3022-1 AssayMax Swine Prothrombin ELISA Kit (Plasma, Serum, and Cell Culture samples)