AssaySense

Human Alpha Thrombin Chromogenic Activity Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 10 µl of Standard or Sample per well.
Add 90 µl of AssayMix per well.
Read at 405 nm at 0 minutes. Incubate at 37°C.

Step 2. Read every 30 minutes for 2 hours at 405 nm.

Symbol Key

Consult instructions for use

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
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6								
7								
8								
9								
10								
11								
12								

Human Alpha Thrombin Chromogenic Activity Kit

Catalog No. CT4010

Sample insert for reference use 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 Assay

The AssaySense Human Alpha Thrombin Chromogenic Activity Kit is developed to determine human thrombin activity in **plasma, serum, and cell culture samples**. The amidolytic activity of thrombin is quantitated using a highly specific thrombin substrate releasing a (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the thrombin enzymatic activity.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents 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 insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **Microplate:** One 96-well polystyrene microplate (12 strips of 8 wells).
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.

- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Human Thrombin Standard:** 1 vial, lyophilized (0.48 AU)
- **Thrombin Substrate:** 2 vials, lyophilized

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard and Thrombin Substrate at -20°C.
- Store Microplate and EIA Diluent (10x) at 2-8°C.
- Unused microplate wells may be returned and resealed.
- Diluted EIA Diluent (1x) may be stored for up to 30 days at 2-8°C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes, use supernatants. Dilute samples 1:2 with EIA and assay. If necessary, dilute samples within the range of 1x – 10x into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, remove serum. Dilute samples 1:20 with EIA and assay. If necessary, dilute samples within the range of 5x – 50x into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris and collect supernatants. If necessary, dilute samples into EIA Diluent 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 Human Thrombin Standard (0.48 AU) with 0.3 ml of EIA Diluent to generate a 1.6 AU/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 standard solution (1.6 AU/ml) 1:2 with EIA Diluent to produce 0.8, 0. 4, 0.2, 0.1, and 0.05 AU/ml solutions. EIA Diluent serves as the zero standard (0 AU/ml). Any remaining solution should be frozen at -20°C and used within 15 days.

Standard Point	Dilution	Thrombin (AU/ml)
P1	Standard (1.6 AU/ml)	1.60
P2	1 part P1 + 1 part EIA Diluent	0.80
P3	1 part P2 + 1 part EIA Diluent	0.40
P4	1 part P3 + 1 part EIA Diluent	0.20
P5	1 part P4 + 1 part EIA Diluent	0.10
P6	1 part P5 + 1 part EIA Diluent	0.05
P7	EIA Diluent	0.00

- **Thrombin Substrate**: Add 1 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Assay Procedure

- Add 10 μl of Thrombin Standard or sample per well.
- AssayMix: Freshly prepare the desired volume of the AssayMix by combining the following reagents according to the assay numbers (n)**.**

Assay Mix Reagents	n=1
EIA Diluent (1x)	70 μl
Thrombin Substrate	20 μl

- Add 90 μl of the above AssayMix to each well and tap plate to mix gently.
- Read at 405 nm at 0 minutes.
- Incubate at 37°C, and read the absorbance at 405 nm every 30 minutes for 2 hours.

Thrombin Standard or Sample	10 µl
AssayMix	90 µl
Read at 405 nm at 0 minutes. Cover wells with sealing tape. Incubate 37°C. Read the absorbance at 405 nm every 30 minutes for 2 hours.	

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

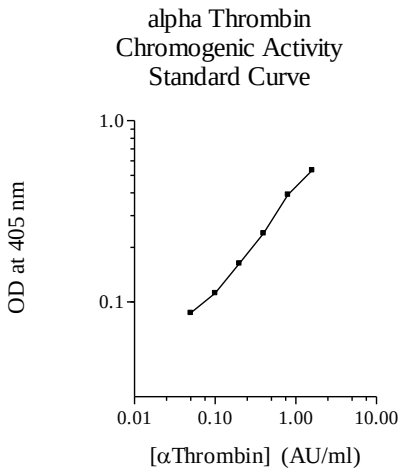
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	AU/ml	Average OD
P1	1.60	0.584
P2	0.80	0.429
P3	0.40	0.262
P4	0.20	0.171
P5	0.10	0.118
P6	0.05	0.089
P7	0.00	0.059
Sample: Pool Normal, Sodium Citrate Plasma (2x)		0.303

Standard Curve

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



Reference Value

- Human Plasma from healthy adults were tested (n=20). On average, thrombin level was 1.05 AU/ml.

Sample	n	Average Value (AU/ml)
Human Pool Normal Plasma	10	1.2
Human Normal Plasma	10	0.9

Performance Characteristics

- The minimum detectable dose of thrombin as calculated by 2SD from the mean of a zero standard was established to be 0.02 AU/ml.
- No significant cross-reactivity or interference was observed.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	2.0%	2.3%	3.4%	7.0%	7.8%	6.5%
Average CV (%)	2.6%			7.1%		

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

	Average Percentage of Expected Value (%)	
Sample Dilution	Plasma	Serum
No Dilution	104%	102%
1:2	97%	96%
1:4	101%	94%

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 1.0R

Related Products

- ET4010-1 AssayMax Human Thrombin ELISA Kit (Cell Culture samples)
- 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)