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ab234620 Thrombin Activity Assay

For the measurement of thrombin activity in human plasma, serum, cell culture, cell lysate, and tissue samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Thrombin Activity Assay (ab234620) is developed to determine thrombin activity in human 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.

Prepare all reagents, samples and standards as instructed.

Add 10 µL of standard or sample to each well.

Add 90 µL of Assay Mix per well.

Read absorbance at 405 nm at zero minutes for background reading. Cover and incubate at 37°C.

Read absorbance every 30 minutes for 2 hours.

2. Materials Supplied and Storage

- Store kit at 4-8°C or -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Reconstituted components are stable for 1 month.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Microplate	1 unit	4°C
Sealing Tapes	3 units	4°C
10X Diluent M Concentrate	20 mL	4°C
Human Thrombin Standard (Lyophilized)	1 vial	-20°C
Thrombin Substrate (Lyophilized)	2 vials	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 405 nm.
- Deionized or distilled reagent grade water.
- Incubator (37°C).

4. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Prepare all reagents as instructed prior to running the assay. Freshly dilute all reagents and bring all reagents to room temperature before use.

5.1 10X Diluent M Concentrate

1. Dilute the Diluent Concentrate 10-fold with reagent grade water to produce a 1X solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle.
2. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.

5.2 Human Thrombin Standard

1. Reconstitute the Human Thrombin Standard (0.73 AU) with 0.45 mL of Diluent to generate a 1.6 AU/mL (0.2 WHO U/mL) standard stock solution.
2. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions.
3. Any remaining stock solution should be stored at -20°C and used within 30 days.

5.3 Thrombin Substrate

1. Add 1.4 mL of reagent grade water to produce a 1X stock solution.
2. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice.
3. Any remaining stock solution should be stored at -20°C and used within 30 days.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (1.6 AU/mL) 2-fold with equal volume of Diluent to produce 0.8, 0.4, 0.2, 0.1, and 0.05 AU/mL solutions. Diluent serves as the zero standard (0 AU/mL).

7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay. Avoid repeated freeze-thaw cycles.

7.1 Plasma

1. Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant.
2. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma.
3. A 2-fold sample dilution is suggested into Diluent or within the range of 1X– 10X; however, user should determine optimal dilution factor depending on application needs.
4. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
   Δ Note: EDTA or Heparin can also be used as an anticoagulant.

7.2 Serum

1. Samples should be collected into a serum separator tube.
2. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum.
3. A 20-fold sample dilution is suggested into Diluent or within the range of 5X – 50X; however, user should determine optimal dilution factor depending on application needs.
4. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

7.3 Cell Culture Supernatants

1. Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatants.
2. If necessary, dilute samples into Diluent; user should determine optimal dilution factor depending on application needs.
3. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

7.4 Cell Lysate

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 106 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

7.5 Tissue

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

1. Prepare all reagents, standard solutions and samples for initial setup as instructed. The assay is performed at 37°C in a humid incubator.
2. Remove excess microplate strips from the plate frame.
3. Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one well. It is recommended that Assay Mix be made in 10% excess.

Assay Mix Reagents	n = 1 well
Diluent (1X)	65 µL
Thrombin Substrate	25 µL

4. Add 10 µL of Human Thrombin Standard or sample to each well. Gently tap plate to thoroughly coat the wells.
5. Add 90 µL of Assay Mix to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
6. Read the absorbance at 405 nm for a zero minute background reading. Cover the wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation. Incubate microplate at 37°C after each reading.
7. Read the absorbance at 405 nm every 30 minutes for 2 hours. Cover wells with a sealing tape and incubate at 37°C after each reading.

9. Data Analysis

Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve from the optimal reaction time plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance (OD) or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter curve. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

10. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

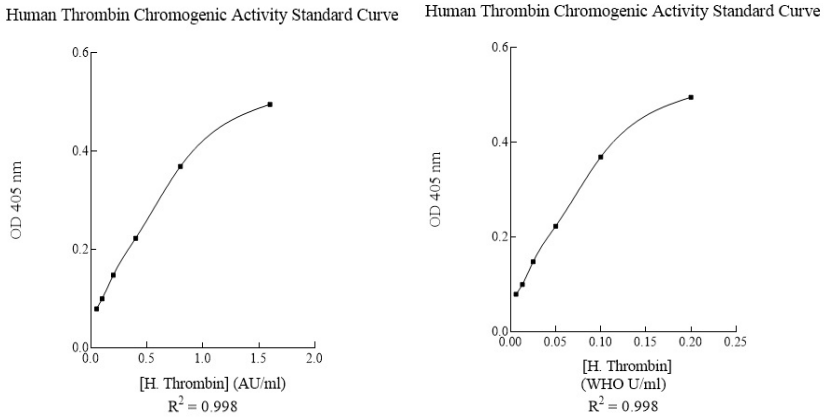

Figure 1. Human Thrombin Chromogenic Activity Standard Curve.

SENSITIVITY

The minimum detectable dose of human thrombin as calculated by 2SD from the mean of a zero standard was established to be 0.034 AU/ml.

11. Troubleshooting

Problem	Cause	Solution
Low precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

Problem	Cause	Solution

Problem	Cause	Solution
Unexpectedly low or high signal intensity	Microplate was left unattended between steps.	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Problem	Cause	Solution
Deficient standard curve fit	Non-optimal sample dilution	User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)