Thrombin Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1328

(Version 1.1A)

Detection and Quantification of Thrombin Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture supernatant and Other biological fluids.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Thrombin is the central executor of the coagulation cascade, converting soluble fibrinogen into insoluble fibrin to directly drive thrombus formation. Precise measurement of its activity is a key biochemical parameter for evaluating coagulation function, diagnosing bleeding or thrombotic disorders, and monitoring anticoagulation therapy.

Thrombin Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring thrombin activity in a variety of samples. In this colorimetric assay, 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.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	18 ml x 1	4 °C
Substrate	Powder x 2	-20 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Positive Control Diluent	4 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
Standard Diluent	1 ml x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Standard Diluent to generate 10 mmol/L of standard stock solution, store at -20 °C for 1-2 weeks after reconstitution. Dilute to 2 mmol/L top standard solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Then perform 2-fold serial dilutions of the top standard solution by distilled water to make the standard curve. The concentration of standard curve could be 2000/1000/500/250/125/62.5/31.25 µmol/L. Prepare immediately before use.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to 1 vial then vortex before use, store at 4°C for 1-2 days or -20 °C for 2-3 weeks.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 ml Positive Control Diluent to generate 10× stock solution. Dilute the stock solution 10-fold using Positive Control Diluent to prepare the Positive Control working solution (eg. 10 µl to 90 µl Positive Control Diluent). Store at 4°C for 2-3 days or -20 °C for 1-2 months or -80 °C for 6 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell samples

Collect cell into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	160 µl	160 µl	160 µl	160 µl	160 µl
Sample	20 µl	--	--	--	--
Distilled Water	--	20 µl	--	--	--
Positive Control	--	--	20 µl	--	--
Substrate	20 µl	20 µl	20 µl	--	--
Mix, put it into the convection oven, 37 °C for 60 minutes.					
Standard	--	--	--	40 µl	--
Distilled water	--	--	--	--	40 µl
Record absorbance measured at 405 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the incubation time to 120 minutes; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VII. CALCULATION

Unit Definition: One unit of Thrombin activity is defined as the enzyme will catalyze the hydrolysis of substrate and release 1 μmol p-nitroaniline(pNA) per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \quad (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T} \quad (U/mg)$$

2.2 According to the quantity of cells

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \quad (U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \quad (U/g)$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \quad (U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

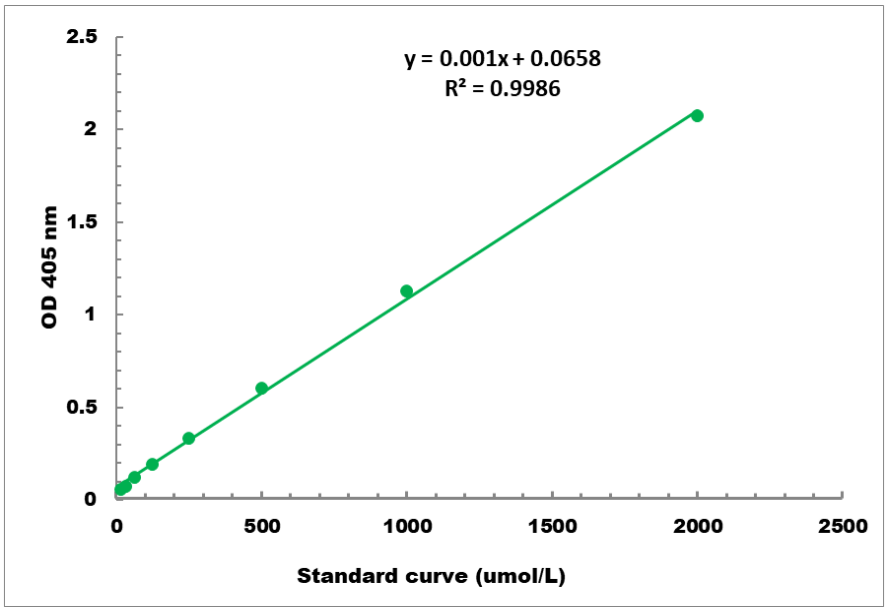
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

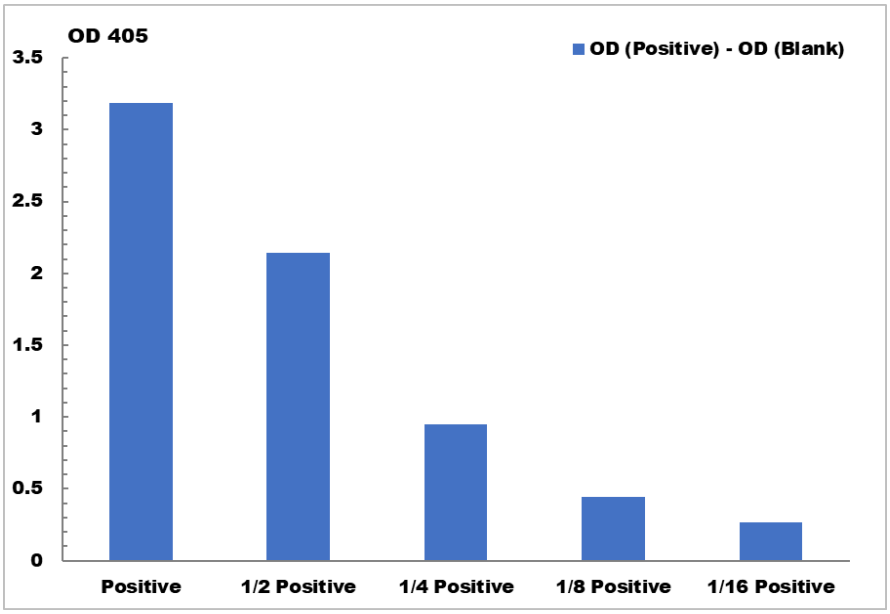
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 20 µmol/L - 2000 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration