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# Product Information

**Thrombin Activity Fluorometric Assay Kit**

Catalog Number **MAK242**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Thrombin (EC 3.4.21.5, Factor IIa), a serine protease, is an important clotting factor in the coagulation cascade that involves the conversion of soluble fibrinogen to insoluble active fibrin strands. In this pathway, prothrombin is proteolytically converted into active thrombin.

Thrombin is also a potent vasoconstrictor and mitogen implicated as a major factor in vasospasm following subarachnoid hemorrhage. Ruptured cerebral aneurysm blood clots around a cerebral artery releases thrombin, which in turn induces acute and prolonged narrowing of the blood vessel, potentially resulting in cerebral ischemia and infarction (stroke). In addition, it is a pro-inflammatory enzyme that may influence the onset and progression of atherosclerosis.

This Thrombin Activity Fluorometric Assay Kit utilizes the ability of thrombin to proteolytically cleave a synthetic substrate and release a fluorophore, AMC, which can be easily quantified by fluorescence. This assay kit is simple, rapid, and can detect thrombin activity as low as 1 ng in samples.

### Components

The kit is sufficient for 100 assays in 96 well plates.

| | |
|---|---|
| Thrombin Dilution Buffer<br>Catalog Number MAK242A | 1 mL |
| Thrombin Assay Buffer<br>Catalog Number MAK242B | 15 mL |
| Thrombin Enzyme Standard<br>Catalog Number MAK242C | 5 µL |
| Thrombin Stubstrate<br>Catalog Number MAK242D | 0.5 mL |

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – white plates are recommended for this assay.
- Fluorescence multiwell plate reader

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

Thrombin Assay Buffer – Bring to room temperature before use.

Thrombin Enzyme Standard – Prepare a stock solution of Thrombin Enzyme (50 ng/µL) by adding 12 µL of Thrombin Dilution buffer to 4 µL of Thrombin Enzyme Standard. Mix, aliquot, and store at –80 °C. Avoid repeated freeze/thaw cycles.

### Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

**Procedure**
Read entire protocol before performing the assay.

Sample Preparation
Add 2–50 μL of sample containing thrombin per well of 96 well plate and adjust the volume to 50 μL with Thrombin Assay Buffer.

Standard Curve Preparation
Dilute Thrombin Enzyme Standard to 2.5 ng/μL by adding 38 μL of Thrombin Dilution Buffer to 2 μL of Thrombin Enzyme stock solution (50 ng/μL). Mix and add 0, 2, 4, 6, 8 and 10 μL of diluted Thrombin Enzyme Standard (2.5 ng/μL) into a series of wells in a 96 well plate. Adjust the volume to 50 μL with Thrombin Assay Buffer to prepare 0, 5, 10, 15, 20 and 25 ng/well of Thrombin Enzyme Standard.

Note: Store the diluted Thrombin Enzyme Standard solution at –80 °C.

Substrate Mix
Prepare enough reagents for the number of assays to be performed. Prepare 50 μL of Substrate Mix for each standard and sample well, see Table 1.

**Table 1.**
Preparation of Substrate Mix

| Reagents | Volume |
|---|---|
| Thrombin Assay Buffer | 45 μL |
| Thrombin Substrate | 5 μL |

Mix and add 50 μL of Thrombin Substrate Mix into each standard and sample well, and mix well.

Measurement
Measure fluorescence in kinetic mode for 30–60 minutes at 37 °C ($\lambda_{ex}$ = 350 nm/$\lambda_{em}$ = 450 nm). Choose two time points ($T_1$ & $T_2$) in the linear range of the plot and obtain the corresponding values for the fluorescence ($RFU_1$ and $RFU_2$).

**Results**
Calculations
Apply sample's ΔRFU to to obtain corresponding

Subtract 0 Standard reading from all readings. Plot the Thrombin Standard Curve. Apply the ΔRFU of the sample to the Thrombin Standard Curve to obtain corresponding Thrombin (B, in ng) and calculate the activity of Thrombin in the sample as:

$$\text{Fxa Activity } \left(\frac{\text{ng/mL}}{\text{μg/L}}\right) = \frac{B}{V} \times \text{dilution factor}$$

B = Thrombin amount from Standard Curve (ng)
V = sample volume added into the reaction well (mL)

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay Not Working | Cold assay buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | White plates are recommended for this assay. |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if samples will be used multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Reaction Mix before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the reaction mix |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Reaction Mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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