

Diagnostic relevance

The conversion of prothrombin to thrombin is a key event in thrombus (clot) formation and perpetuation of the clotting process. Thrombin is a serine protease that induces a variety of enzymatic and cellular reactions, including conversion of fibrinogen into a fibrin clot, activation of the cofactor proteins V and VIII, and activation of platelets and endothelial cells. The activity of thrombin is tightly regulated by several endogenous inhibitory mechanisms, such as the antithrombin–heparin pathway and the protein C pathway.

Clinically, impaired or unregulated thrombin formation predisposes patients to enhanced bleeding or to the development of thromboembolic complications.

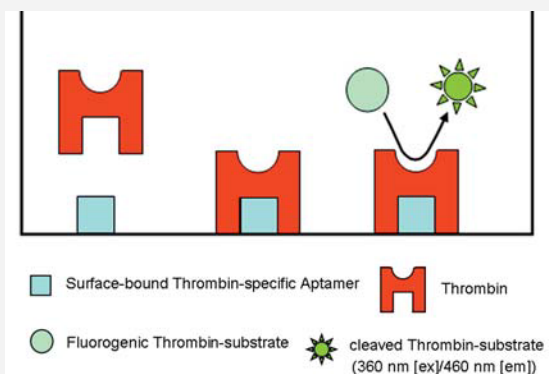
However, no diagnostic assay is available to date that enables the direct measurement of thrombin concentrations in circulating blood from patients. In vivo generated thrombin can be indirectly assessed by the measurement of prothrombin fragment F1.2, an activation peptide generated during the conversion of prothrombin to thrombin, or thrombin-antithrombin-complexes (TAT), formed during the inactivation of thrombin by its major plasma inhibitor antithrombin. However, due to differential accumulation in the circulation, these parameters do not reflect the current state level of functional active thrombin in vivo. The assays rather provide integrated information on recent thrombin formation.

OLIGOBIND® Thrombin activity assay

The newly developed OLIGOBIND® Thrombin assay (product no. ADG844) allows the **direct quantification of functional active thrombin in plasma from peripheral blood**.

To prevent inactivation of thrombin prior to analysis, special Thrombin-blood collection tubes (product no ADG844T) are used for blood drawing.

Principle of the OLIGOBIND® Thrombin activity assay



References

- Profiling of active thrombin in human blood by supramolecular complexes. Müller J, Becher T, Braunstein J, Berdel P, Gravius S, Rohrbach F, Oldenburg J, Mayer G, Pötzsch B. Angew Chem (Int Ed Engl) 2011 Jun 27;50(27):6075-6078.
- Measurement of free thrombin in human plasma using an oligonucleotide-based enzyme capture assay (OECA). Becher T, Müller J, Braunstein J, Mayer G, Pötzsch B. Haemostasiologie, 2010, 30 1: P17-02

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Product information OLIGOBIND® Thrombin assayover

OLIGOBIND[®] Thrombin activity assay, ADG844



Description

The OLIGOBIND[®] Thrombin Assay is performed in 96-well microtiter plate format. Immobilized HD1-22, a bivalent and high-affinity thrombin-binding DNA aptamer, is used to capture thrombin from plasma samples. Since HD1-22 binds thrombin through both anionic exosites the active center is not occupied. This allows quantification of aptamer bound thrombin through hydrolysis rates of a fluorogenic peptide substrate.

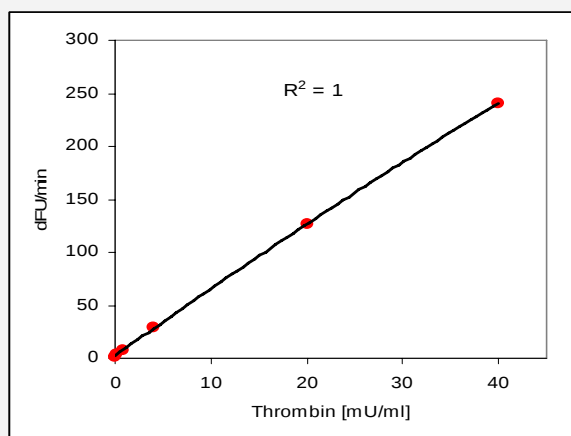
To prevent inactivation of thrombin prior to analysis, special Thrombin-blood collection tubes are needed for blood drawing. Total assay time is less than two hours, allowing the utilization of the OLIGOBIND[®] Thrombin in daily routine practice.

Thrombin levels in plasma were found to be elevated during major surgery. The amount of thrombin formation correlated with the surgical trauma. Taken together, the OLIGOBIND[®] Thrombin assay is a powerful tool that allows sensitive measurement of free thrombin in human plasma.

Principle of the assay

Stabilized plasma samples are added to microwells coated with a DNA-aptamer against thrombin. During an incubation period, thrombin present in the sample will bind to the aptamer coated to the wells. Following a washing step, a fluorogenic peptide substrate for thrombin is added to the microwells. Measuring the change of fluorescence ($360_{[ex]}/460_{[em]}$ nm) and extrapolating the value with those of a standard curve determines the level of thrombin in the plasma sample.

Standard curve



Key Features

- **Format:** 96-well plate
- **Pre analytic:** Special blood collection tubes (Product no. ADG844T)
- **Sample Vol.:** 200 µl (duplicate analysis)
- **Assay Range:** 0 - 40 mU/ml
- **Assay time:** < 2 hours
- **Precision:** intra-assay: $6.9 \pm 0.6 \%$
inter-assay: $6.5 \pm 3.1 \%$

Order numbers

OLIGOBIND[®] Thrombin assay ADG844

Thrombin-blood collection tubes ADG844T

REFER TO APPROPRIATE PACKAGE INSERT FOR ADDITIONAL TECHNICAL INFORMATION

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