

## Introduction

The Thrombin-Receptor (PAR-1, F2R) is a seven transmembrane, G-protein- coupled receptor which is located in thrombocytes and endothelial cells. These enzymes cleave the N-terminus of the receptor, which in turn acts as a tethered ligand. In the cleaved state, part of the receptor itself acts as the agonist, causing a physiological response. The occurrence of auto-antibodies against thrombin receptor is associated with the existence of different vascular diseases.

The CellTrend anti-Thrombin-Receptor - Antibody-EIA is designed for the determination of antibodies (IgG) against the Thrombin-Receptor 1 in serum.

## Principle of the Assay

The CellTrend anti PAR-1 antibody EIA is an antibody screening test. PAR-1 has been pre-coated onto a microtiter plate. During the first incubation the anti-PAR-1 antibodies of the samples are immobilised on the plate. The autoantibodies are detected with a HRP labeled anti-human IgG antibody. In the following enzymatic substrate reaction the intensity of the colour correlates with the concentration and/ or avidity of anti-PAR-1 antibodies

## Precautions

Store the kit at 2-8 °C.

For in vitro use only.

For research use only

Do not use the reagents beyond the expiration date marked on box label.

Please read the instructions carefully before using the kit.

The assay procedure should be carried out only by qualified and well trained employees.

Lipaemic, icteric, haemolysed or microbially contaminated specimen may cause interference.

Do not mix reagents from different lots.

Some components of the kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

Some components of this kit contain ProClin 300. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see materials provided). Wash thoroughly with water in case of contact and possibly look up a doctor.

The stop solution contains 0.5 M sulphuric acid. Wash thoroughly with water in case of contact with skin. In case of contact with eyes rinse with much water and look up a doctor.

Do not allow the wells to become dry once the assay has begun.

## Other supplies required

Deionized or distilled water  
Graduated cylinder  
Micropipettes, multipipette  
Microplate shaker  
Microplate reader  
Refrigerator (2-8 °C)

## Preparation of reagents and samples

- Bring all reagents to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips [MTP] are ready to use. Remove excess strips (breakable) from the frame, reseal in the bag with the desiccant and store at 2-8 °C.

- Dilute the wash buffer [BUF|WASH|10x] with deionized or distilled water **1:10** (e. g. 50 ml + 450 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Dilute the HRP conjugate [CONJ|ENZ100x] with diluent [DIL|Conj] **1:100** (e. g. 50 µl + 4950 µl diluent [DIL|Conj]). The required amount of conjugate solution should be prepared freshly.

- Dilute the human serum or plasma samples with diluent [DIL|SPE] **1:100** (e. g. 5 µl + 495 µl diluent). Store undiluted samples at -20 °C.

- Standards [CAL|1-6], control [CONTROL|1], the diluent sample [DIL|SPE] and the diluent conjugate [DIL|Conj], are ready to use.

## Assay procedure

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous section.
2. Pipette 100 µl of diluted samples, standards, control or diluent [DIL|SPE] (as blank, zero standard) into the wells.
3. Seal wells with adhesive strip and incubate for 2 hours at 4°C temperature.
4. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Dispense 100 µl of diluted HRP conjugate into each well
6. Seal wells with adhesive strip and incubate for 1 hour (with shaking) at room temperature.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate [SUBS|TMB] solution into each well.
9. Incubate for 20 minutes at room temperature in the dark.
10. Add 100 µl of stop solution [SOLN|STOP] to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

### Calculation of results

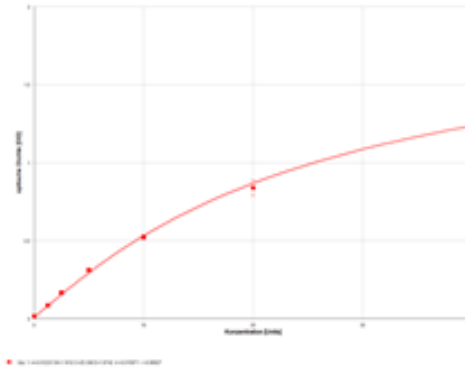
Create a standard curve using computer software capable of generating a curve fit (four parameter fit; x-axis: linear, anti-PAR-1-Ab standard points (1.25 U/ml, 2.5 U/ml, 5 U/ml, 10 U/ml, 20 U/ml, 40 U/ml), use the blank as zero standard 0 U/ml; y-axis: linear, absorbance). The sample concentrations can be calculated from the standard curve.

A run is considered valid if the control is in the expected range (see label).  
Samples > 4.2 U/ml are positive, samples < 4.2 U/ml are negative.

We recommend that each laboratory establish its own cut off for the population tested.

Samples over the standard curve can be assayed again using a higher dilution factor (e.g. 1:500). In this case the concentration read from the standard curve must be multiplied by the additional dilution factor (e.g. 5 for 1:500 dilution).

### Typical data



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

### Precision:

- Intra-assay precision (CV)  
(n=10)  
Sample 1 (5.6 Units): 7.8%

- Inter-assay precision (CV)  
(n=10)  
Sample 1 (7.1 Units): 9.1%

### INSTRUCTIONS FOR USE

## EIA for Quantitative Determination of Anti-PAR-1 Antibodies

**REF** 12200



For Research Use Only

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**Materials provided:**

MTP			Microplate strips, Thrombin-Receptor (PAR1) coated	12 x 8
BUF	WASH	10x	Wash buffer, 10fold conc. ◆	50 ml
DIL	SPE		Diluent sample, ready to use ◆	50 ml
DIL	Con		Diluent conjugate, ready to use ◆	14 ml
CAL	1-6		Standards, ready to use [1.25 - 2.5 - 5 - 10 - 20 - 40 U/ml] ◆	1 ml
CONTROL	1		Control, ready to use ◆	1 ml
CONJ	ENZ	100x	anti-human-IgG, HRP conjugate, 100fold conc. ◆	0.3 ml
SUBS	TMB		TMB substrate, ready to use	12 ml
SOLN	STOP		Stop solution, ready to use (0.5 M sulphuric acid)	12 ml

◆: contains ProClin 300

**Assay procedure summary:**A. Preparation

1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Dilute samples with diluent sample 1:100
4. Dilute freshly HRP conjugate 1:100 with diluent conjugate

B. Performance

1. Pipette 100 µl of samples, standards, control into the wells
2. Incubate for 2 hours at 4°C temperature
3. Wash three times with 300 µl of wash buffer
4. Dispense 100 µl of HRP conjugate solution
5. Incubate for 1 hour (with shaking) at room temperature
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 20 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

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**Symbols / Symbole / Symbôles / Simbolos / Simbolos / Simboli / Συμβολα**

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro .ιάγνωση.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / .ιαβάστε τις οδηγίες πριν την χρήση.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:

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	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro .ιάγνωση.
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