

## Introduction

Endothelins (ET) are 21-amino acid vasoconstricting peptides produced primarily in the endothelium having a key role in vascular homeostasis. It mediates the effects through G-Protein-coupled receptors, the Endothelin receptors. There are two key receptor types, ET<sub>A</sub> and ET<sub>B</sub>. ET<sub>A</sub> receptors are found in the smooth muscle tissue of blood vessels where they increase vasoconstriction by ET-1.

The CellTrend anti-Endothelin Receptor A Antibody-EIA is designed for the determination of antibodies (IgG) against the Endothelin receptor subtype A in human serum and plasma.

## Principle of the Assay

The CellTrend anti-Endothelin Receptor A Antibody-EIA is an antibody screening test. Endothelin- receptor A has been pre-coated onto a microtiter plate. During the first incubation the anti-Endothelin receptor A-Antibodies of the samples are immobilised on the plate. The autoantibodies are detected with a POD 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-Endothelin receptor A-antibody.

## Precautions

Store the kit at 2-8 °C (shipping to end user at room temperature).

For Research Use Only! Not for diagnostic use.

For in vitro use only.

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

Do not mix reagents from different lots.

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.

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.

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)

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:
	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: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:

## References

1. Gabriela Riemekasten, Aurélie Philippe, Melanie Näther, Torsten Slowinski, Dominik N Müller, Harald Heidecke, Marco Matucci-Cerinic, László Czirják, Ivo Lukitsch, Mike Becker, Angela Kill, Jacob M van Laar, Rusan Catar, Friedrich C Luft, Gerd R Burmester, Björn Hegner, Duska Dragun: *Involvement of functional autoantibodies against vascular receptors in systemic sclerosis*. Ann Rheum Dis 2011 Mar; 70(3): 530-536

## Patents

Protected under U.S. Patent No. 8,592,164

## INSTRUCTIONS FOR USE

### Endothelin Receptor A (ETAR) Antibody EIA

**REF** 12105 / EIA-ETARX



**For research use only. Not for use in diagnostic procedures.**

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## 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, controls or diluent **[DIL | SPE]** (as blank) into the wells.
3. Seal wells with adhesive strip and incubate for 2 hours at 2-8°C temperature.
4. Remove 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

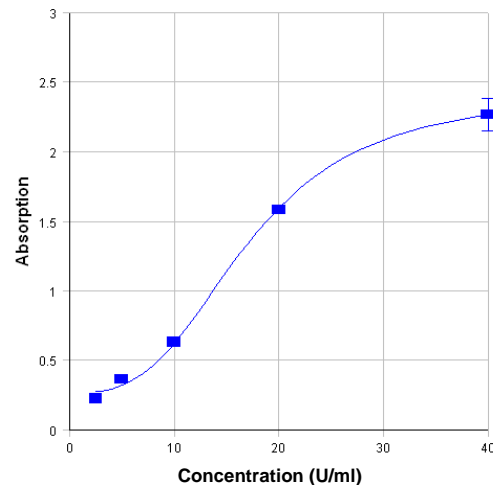
Create a standard curve using computer software capable of generating a curve fit (four parameter fit; x-axis: linear, anti-ETAR-Ab standard points (2.5 U/ml, 5 U/ml, 10 U/ml, 20 U/ml, 40 U/ml); y-axis: linear, absorbance). The sample concentrations can be calculated from the standard curve.

A run is considered valid if the positive control is in the expected range (see label) and the negative control is less than the cut off (10 U/ml).

Samples >17 U/ml are positive, samples 10-17 U/ml are at risk. Samples <10 U/ml are negative. 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).

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

## 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 (21.4 U/ml): 6.3%

- Inter-assay precision (CV)

(n=20)

Sample 1 (22.9 U/ml): 8.3%

## Materials provided:

MTP			Microplate strips, Endothelin Receptor A coated	12 x 8
BUF	WASH	10x	Wash buffer, 10fold conc. ◆	50 ml
DIL	SPE		Diluent sample, ready to use ◆	50 ml
DIL	Conj		Diluent conjugate, ready to use ◆	14 ml
CAL	1-5		Standards, ready to use [2.5 - 5 - 10 - 20 - 40 U/ml] ◆	1 ml
CONTROL	+		Positive control, ready to use ◆	1 ml
CONTROL	-		Negative control, ready to use ◆	1 ml
CONJ	ENZ	100x	anti-human-IgG, HRP conjugate, 100fold conc. ◆	0.2 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, controls into the wells
2. Incubate for 2 hours at 2-8°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

## Sample Collection and Storage

Collect serum or plasma according to your standard procedure.

Store at room temperature up to 48 hrs.

Store at 2-8 °C up to 4 days

Long term storage up to 12 month at - 20 °C.  
Avoid repeated freeze-thaw cycles.

## 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 ENZ 100x** 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).

- Standards **CAL 1-5**, positive control **CONTROL +**, negative control **CONTROL -**, the diluent sample **DIL SPE**, the diluent conjugate **DIL Conj**, the substrate **SUBS TMB**, and the stop solution **SOLN STOP** are ready to use.