

## Introduction

The  $\beta$ 1-adrenergic receptor is a G protein-coupled receptor.  $\beta$  receptors have the subtypes  $\beta$ 1,  $\beta$ 2 and  $\beta$ 3. Agonist binding causes a rise in the intracellular concentration of the second messenger cAMP. The occurrence of auto-antibodies against the  $\beta$ 1-adrenergic receptor is associated with the existence of dilated cardiomyopathy (DCM). The CellTrend anti- $\beta$ 1-adrenergic receptor antibody EIA is designed for the determination of antibodies (IgG) against the  $\beta$ 1-adrenergic receptor in serum and plasma.

## Principle of the Assay

The CellTrend anti- $\beta$ 1-adrenergic receptor antibody EIA is an antibody screening test.  $\beta$ 1-adrenergic receptors has been pre-coated onto a microtiter plate. During the first incubation the anti- $\beta$ 1 adrenergic receptor 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- $\beta$ 1-adrenergic receptor antibodies

## Precautions

Store the kit at 2-8 °C.

For in vitro use only.

Outsite EU: 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 reader

## 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  $\mu$ l + 4950  $\mu$ l 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  $\mu$ l + 495  $\mu$ l diluent). Store undiluted samples at -20 °C.

- Standards [CAL | 1-5], positive control [CONTROL | +], negative control [CONTROL | -], 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  $\mu$ l 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 4°C temperature.
4. Aspirate fluid from wells and wash three times with 300  $\mu$ l wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Dispense 100  $\mu$ l 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  $\mu$ l of TMB substrate [SUBS | TMB] solution into each well.
9. Incubate for 20 minutes at room temperature in the dark.
10. Add 100  $\mu$ l 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- $\beta$ 1 adr-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.

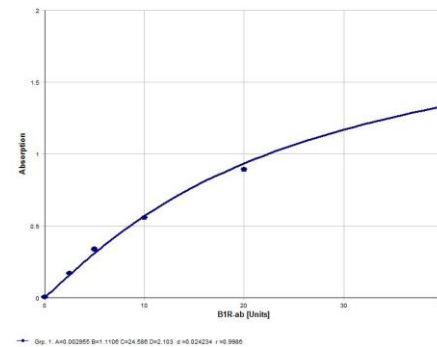
A run is considered valid if the positive control is in the expected range (see label) and the negative control is less than the cut off (15 U/ml).

Samples  $\geq 15$  U/ml are positive, samples  $< 15$  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).

Therapy should not be decided based on results alone. The results should be correlated to other clinical observations and diagnostic tests. Furthermore, 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

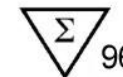
- Intra-assay precision (CV)  
Sample: 3.69%

- Inter-assay precision (CV)  
Sample: 5.11%

### INSTRUCTIONS FOR USE

## EIA for Quantitative Determination anti- $\beta$ 1-adrenergic receptor ( $\beta$ 1 adr)-Antibodies

**REF** 12600



For USA: Research Use Only



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**Materials provided:**

MTP			Microplate strips, $\beta$ 1 adrenergic-Receptor coated	12 x 8
BUF	WASH	10x	Wash buffer, 10fold conc. $\blacklozenge$	50 ml
DIL	SPE		Diluent sample, ready to use $\blacklozenge$	50 ml
DIL	Conj		Diluent conjugate, ready to use $\blacklozenge$	14 ml
CAL	1-5		Standards, ready to use [2.5 - 5 - 10 - 20 - 40 U/ml] $\blacklozenge$	1 ml
CONTROL	+		Positive control, ready to use $\blacklozenge$	1 ml
CONTROL	-		Negative control, ready to use $\blacklozenge$	1 ml
CONJ	ENZ	100x	anti-human-IgG, HRP conjugate, 100fold conc. $\blacklozenge$	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

$\blacklozenge$ : 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  $\mu$ l of samples, standards, controls into the wells
2. Incubate for 2 hours at 4°C temperature
3. Wash three times with 300  $\mu$ l of wash buffer
4. Dispense 100  $\mu$ l of HRP conjugate solution
5. Incubate for 1 hour (with shaking) at room temperature
6. Wash three times with 300  $\mu$ l of wash buffer
7. Dispense 100  $\mu$ l of TMB substrate solution
8. Incubate for 20 minutes at room temperature in the dark
9. Add 100  $\mu$ l 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: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:

**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.
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