Anti Endothelin Receptor A IgG Antibody ELISA

Catalog Number:
ERG31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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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, ETA and ETB. ETA receptors are found in the smooth muscle tissue of blood vessels where they increase vasoconstriction by ET-1.

The Eagle Biosciences Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit is designed for the determination of antibodies (IgG) against the Endothelin receptor subtype A in serum and plasma.

Principle of the Assay

The Eagle Biosciences Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit 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 immobilized on the plate. The autoantibodies are detected with a POD labelled 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 Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit at 2-8 °C.
- The Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit is for research use only. Not for use in diagnostic procedures.
- Do not use the reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit.
- Do not mix reagents from different lots.
- Lipemic, icteric, hemolyzed 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.
- Some components of this Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit contain Thimerosal, a mercury containing compound. The stop solution contains 0.5 M sulphuric acid. Follow routine precautions for handling hazardous chemicals.
- 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

Preparation of reagents and samples

- Bring all reagents of the Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.
- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the plastic bag with the desiccant and store at 2-8 °C
- Dilute the wash buffer 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 with diluent 1:100 (e. g. 50 µl + 4950 µl diluent). The required amount of conjugate solution should be prepared freshly.
- Standards are ready to use:

Standard	Conc. (U/ml)
S 5	40
S 4	20
S 3	10
S 2	5.0
S 1	2.5

- Dilute the samples with diluent using 1:100 dilution (eg. 5 µl serum or plasma + 495 µl diluent). If samples generate values outside the standard curve, the dilution factor may be quite varied. Store the undiluted samples at room temperature for 48 hours, 2-8°C 4-days, and long-term storage for up to 12 months at –20 °C. avoid repeated freeze-thaw cycles.

Assay procedure

It is recommended that all samples and standards of the Human Anti Endothelin Receptor A IgG Antibody ELISA Assay Kit 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 (as blank) into the wells.
3. Seal wells with adhesive strip and incubate for 2 hours at 2-8°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 solution into each well.

9. Incubate for 20 minutes at room temperature in the dark.
10. Add 100 µl of stop solution to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

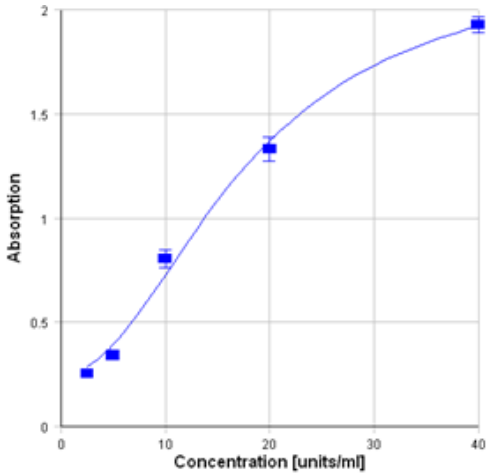
Calculation of results
Create a standard curve using computer software capable of generating a curve fit (four parameter fit; x-axis: linear, anti-ETAR-Ab standard points (2.5 U/ml, 5 U/ml, 10 U/ml, 20 U/ml, 40 U/ml); y-axis: linear, absorbance). The sample concentrations can be calculated from the standard 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 higher 17 U/ml are positive, samples higher 10 U/ml are at risk. Samples less than 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).

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 Standard Curve



Performance Characteristics

- *Standard curve:*
  5 standards between 2.5 U/ml and 40 U/ml
- *Cut off:*
  10 U/ml (at risk)
  17 U/ml (positive)
- *Sample materials:*
  Serum, Plasma

- Intra-assay precision (CV)
(n=10)
Probe 1 (21,4 U/ml): 6.3%

- Inter-assay precision (CV)
(n=20)
22,9 U/ml: 8.3%

Materials provided:

Microplate strips, Angiotensin II-Receptor typ 1 coated	12 x 8
Wash buffer, 10fold conc. ✦	50 ml
Diluent sample, ready to use ✦	50 ml
Diluent conjugate, ready to use ✦	14 ml
Standards, ready to use [2.5 - 5 - 10 - 20 - 40 U/ml] ✦	1 ml
Positive control, ready to use ✦	1 ml
Negative control, ready to use ✦	1 ml
anti-human-IgG1, HRP conjugate, 100fold conc.✦	0.2 ml
TMB substrate, ready to use	12 ml
Stop solution, ready to use (0.5 M sulphuric acid)	12 ml

✦: contains Thimerosal

Assay procedure summary:
A. Preparation
1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Dilute samples with diluent 1:100
5. Dilute freshly HRP conjugate 1:100 with diluent

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 25 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

References

1. Gabriela Riemekasten, Aurélie Philippe, Melanie Näther, et al. Invovment of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis 2011 Mar; 70(3): 530-536

2. Hiemann NE, Meyer R, Wellnhofer E, Schoenemann C, Heidecke H, Lachmann N, Hetzer R, Dragun D. Non-HLA Antibodies Targeting Vascular Receptors Enhance Alloimmune Response and Microvasculopathy After Heart Transplantation. Transplantation. 2012 Oct 2

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