Rat Anti Angiotensin Receptor 1 IgM Antibody ELISA

Catalog Number:
ARM21-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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Introduction

Angiotensin II is a peptide hormone which causes vasoconstriction, increased blood pressure, and release of aldosterone from the adrenal cortex. It occupies an important role in the Renin-Angiotensin-System (RAS). Angiotensin II mediates the effects through G-Protein-coupled receptors, the Angiotensin receptors. The occurrence of autoantibodies against AT1 receptor is associated with an increased risk of an immunologic rejection after organ transplantation. The presence of AT1 autoantibodies correlate with the existence and course of Scleroderma.

This Rat Anti Angiotensin Receptor 1 IgM Antibody ELISA Assay Kit is designed for the determination of Rat antibodies (IgM) against the Angiotensin II receptor subtype I in serum and plasma.

Principle of the Assay

The Eagle Bioscience's Rat Anti Angiotensin Receptor 1 IgM Antibody ELISA Assay Kit is an antibody screening test. Angiotensin II Receptor has been pre-coated onto a microtiter plate. During the first incubation the rat anti-Angiotensin II receptor 1-IgM-Antibodies of the samples are immobilized on the plate. The autoantibodies are detected with a POD labelled anti-Rat IgM antibody. In the following enzymatic substrate reaction the intensity of the colour correlates with the concentration and/ or avidity of anti-Angiotensin II receptor 1-antibody (IgM).

Precautions

- Store the Rat Anti Angiotensin Receptor 1 IgM Antibody ELISA Assay Kit at 2-8 °C.
- The Rat Anti Angiotensin Receptor 1 IgM 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 Rat Anti Angiotensin Receptor 1 IgM Antibody ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rat Anti Angiotensin Receptor 1 IgM 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 reader

Preparation of reagents and samples

- Bring all reagents of the Rat Anti Angiotensin Receptor 1 IgM 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. 40 ml + 360 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)
S6	40
S 5	20
S 4	10
S 3	5
S 2	2.5
S 1	1.25

- 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 Rat Anti Endothelin Receptor A IgM Antibody 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.

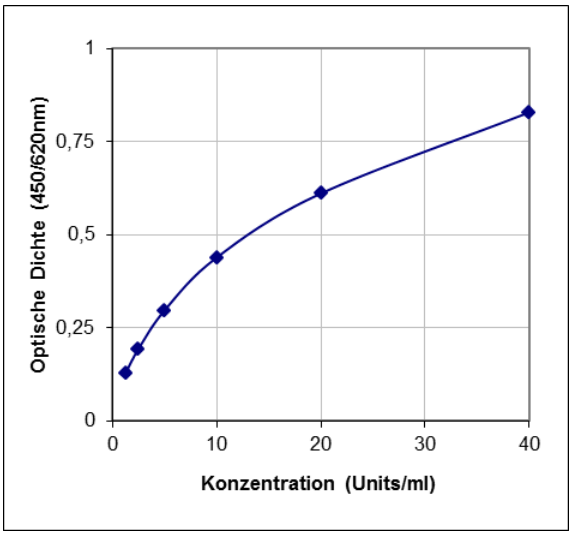
6. Seal wells with adhesive strip and incubate for 1 hour (with shaking) at room temperature.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of TMB substrate solution into each well.
9. Incubate for 20 minutes at room temperature in the dark.
10. Add 100 µl of stop solution to each well.
11. Determine 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-ATR1-Ab standard points (1.25 U/ml, 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.

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 Standard Curve



Performance Characteristics

- *Standard curve:*
  - *6 standards between 1.25 U/ml and 40 U/ml*
- *Sample materials:*
  - *Serum, Plasma*

- *Intraassay-Precision:*
  - *(n=8)*
  - *Probe 1 (17.9 U/ml): 13.4%*

- *Interassay-Precision:*
  - *(n=8)*
  - *19.1 U/ml: 11.2%*

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
anti-Rat-IgM, 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 20 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

References

1. Reinsmoen NL, Lai C-H, Heidecke H, Haas M, Cao K, Ong G, Naim M, Wang Q, Mirocha J, Kahwaji J, Vo AA, Jordan SC, and Dragun D:
*Anti-Angiotensin Type 1 Receptor Antibodies Associated With Antibody Mediated Rejection in Donor HLA Antibody Negative Patients.*Transplantation 2010;90: 1473–1477

2. Kelsch R, Everding AS, Kuwertz-Bröking E, Brand E, Spriewald BM, Sibrowski W, Konrad M, Dragun D:*Accelerated Kidney Transplant Rejection and Hypertensive Encephalopathy in a Pediatric Patient Associated With Antibodies Against Angiotensin Type 1 Receptor and HLA Class II.*Transplantation 2011 Nov 27;92(10):e57-9

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