EAGLE BIOSCIENCES

Anti Aprotinin Antibodies IgG ELISA

Catalog Number:
APR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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Introduction

The Eagle Biosciences Anti-Aprotinin Antibodies IgG ELISA Assay Kit is designed for the quantitative determination of IgG Aprotinin Antibodies in serum samples. The Anti-Aprotinin Antibodies ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

Aprotinin is used in operations to reduce intra-operative and postoperative bleeding tendency. Aprotinin as an allogeneic protein has antigenic properties. It has been shown that formation of IgG antibodies to aprotinin takes place in about 50% of all treated patients. During aprotinin re-exposure allergic reactions may occur due to these preformed anti-aprotinin antibodies. Lethal anaphylactic shocks have been described in literature.

This Anti-Aprotinin Antibodies ELISA Assay Kit allows the detection of IgG antibodies against aprotinin in human serum.

Principle of the Assay

Aprotinin has been pre-coated onto a microtiter plate. During incubation the anti-aprotinin antibodies are immobilized on the plate. For detection of bound antibodies an enzyme-linked anti-human-IgG antibody conjugate is added. After washing off any unbound conjugate, a substrate solution is added. The color developing correlates to the amount of bound antibody conjugate. Absorption at 450 nm is proportional to the concentration and/or avidity of anti-aprotinin antibodies.

Precautions

- Store the Anti Aprotinin Antibodies ELISA Assay Kit at 2-8 °C.
- Do not use the Anti-Aprotinin Antibodies ELISA Assay Kit reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Anti-Aprotinin Antibodies ELISA Assay Kit.
- The Anti Aprotinin Antibodies ELISA Assay procedure should be carried out only by qualified and well trained employees.
- Lipemic, icteric, haemolysed or microbiologically contaminated specimen may cause interference.
- Do not mix reagents from different lots of the Anti-Aprotinin Antibodies ELISA Assay Kit.
- Some components of the Anti-Aprotinin Antibodies ELISA Assay 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 Anti-Aprotinin Antibodies ELISA Assay Kit contain Thimerosal, a mercury containing compound, or sodium azide Follow routine precautions for handling hazardous chemicals. 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.
- Controls and standards containing sodium azide may react with lead and copper plumbing, building explosive metal acids. Flush with sufficient water when disposing of reagents.
- 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 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 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 100x with diluent DIL **1:100** (e. g. 50 µl + 4950 µl diluent). The required amount of conjugate solution should be prepared freshly.
- Dilute the human serum samples with diluent DIL **1:100** (e. g. 5 µl + 495 µl diluent). Store undiluted samples at -20 °C.
- Standards, positive control, negative control, and the diluent 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, controls or diluent (as blank) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room 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 30 minutes 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 15 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.

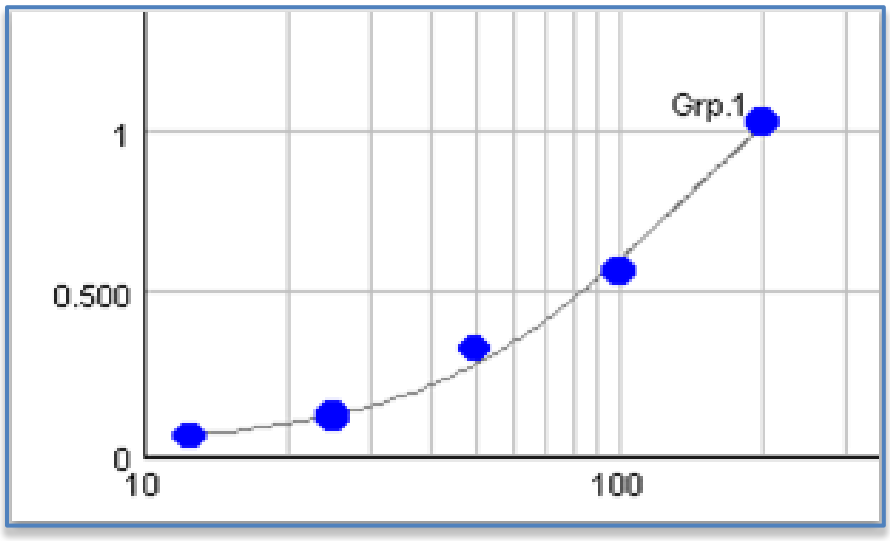
Calculation of results
Create a standard curve for the Anti-Aprotinin Antibodies ELISA Assay using computer software capable of generating a curve fit (four parameter fit; x-axis: log, anti-Aprotinin units; y-axis: linear, absorbance). As an alternative, draw a standard curve on semi-log paper (x-axis: log, anti-Aprotinin units; 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 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).

All precautions in the instructions for use of Aprotinin-containing drugs should be observed even if the test result is negative.

Typical Standard Curve



Performance Characteristics

- *Standard curve: 5 standards between 12.5 U/ml and 200 U/ml*
- *Cut off: 15 U/ml*
- *Sample material: Serum*
- *Intraassay precision (CV):*
  (n=10)
  66 U/ml: 8.7%
  98 U/ml: 4.5%
  195 U/ml: 5.3%
- *Interassay precision (CV):*
  (n=10)
  62 U/ml: 8.9%
  125 U/ml: 15.7%

Materials provided:

Code	Description	Size
MTP	Microplate strips, Aprotinin coated	12 x 8
BUF WASH 10x	Wash buffer, 10fold conc. ◆	50 ml
DIL	Diluent, ready to use ◆	100 ml
CAL 1-5	Standards, ready to use [12.5 - 25 - 50 - 100 - 200 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 Thimerosal
◊ : contains sodium azide

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
4. Dilute freshly HRP conjugate 1:100 with diluent

B. Performance

1. Pipette 100 µl of samples, standards, controls into the wells
2. Incubate for 1 hour at room temperature
3. Wash three times with 300 µl of wash buffer
4. Dispense 100 µl of HRP conjugate solution
5. Incubate for 30 min at room temperature
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 15 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.