Rat Anti-Endothelin 1 ET-1 Polyclonal Antibody ELISA Kit

Catalog #: BG-RAT10903 (96 wells)

User Manual

This kit is designed to quantitatively detect the levels of Rat Anti-Endothelin 1 ET-1 Antibody in cell lysates, serum/ plasma and other suitable sample solution.

Manufactured and Distributed by:

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Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS.

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Intended use

The kit is used to detect the Rat Anti-Endothelin 1 ET-1 Antibody in serum/ plasma and other suitable sample solution.

Assay time	2.0 hours
Validity	Six months
Store at	2-8 °C

Assay principle

The Rat Anti-Endothelin 1 ET-1 Antibody ELISA Kit is based on standard enzyme-linked immunosorbent assay technology. Rat ET-1 has been pre-coated onto 96-well plate. Rat Anti-Endothelin 1 ET-1 Antibody present in the controls/ samples bind to the capture antigen. Subsequently, HRP-conjugated anti-Rat detection antibody is added to form an Ag-Ab complex. After a washing step, the unbound conjugate is removed with wash buffer. Next, addition of HRP substrate, TMB, results in the production of a blue colored product that changes to yellow after the addition of acidic Stop Solution. The density of yellow color is directly proportional to the amount of Rat Anti-Endothelin 1 ET-1 Antibody captured on the plate.

Materials supplied

1. 96-well plate pre-coated with Rat ET-1:	1
2. Sample Diluent buffer :	30 ml
3. Detection antibody-HRP:	12 ml
4. Positive control	1 ml
5. Negative control	1 ml
6. Calibrator	1 ml
7. Chromogen Solution A:	6 ml
8. Chromogen Solution B:	6 ml
9. Stop Solution:	6 ml
10. 20 × Wash Buffer:	25 ml
11. Plate sealers	2
12. Package insert	1

Materials required but not supplied

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Sample dilution

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2- 8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.
Serum/ plasma samples must be diluted 1:100 with the sample diluent buffer.

Preparation of TMB Substrate solution

- The solution should be prepared no more than 10 min prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Mix equal volumes of chromogen solutions A and B. Protect from light.

Wash Buffer

- If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

Assay Procedure

Bring all reagents to room temperature before use. Rat Anti-Endothelin 1 ET-1 Antibody standard curve should be prepared for each experiment.

1. Add 100 µl of sample or controls per well. Add 0.1 ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate at room temperature for 1 hour.
   Note: We recommend that each Rat Anti-Endothelin 1 ET-1 Antibody control and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Repeat the aspiration/wash as in step 2.
4. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate at room temperature for 30 min. Avoid placing the plate in direct light.
5. Repeat the aspiration/wash as in step 2 for three times.
6. Add 100 µl of TMB substrate solution to each well. Cover and incubate at room temperature for 5 -15 min until a gradient develops and you see visible color in the 2nd lowest concentration well. Protect from light. Do not over-develop.
7. Add 50 µl Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Calculation of results

Calculate the mean of duplicate positive control, calibrator and samples.

Calculate anti-ET-1 antibody index of each determination: Divide the mean values of each sample by calibrator mean value

Quality Control

For the assay to be considered valid, the following conditions must be met:

1. Calibrator and Controls must be run with each test run.

2. The OD value of Negative Control must be less than, or equal to 0.3 at 450 nm.

3. If the OD value of the Calibrator is lower than 0.3, the test is not valid and must be repeated.

4. The OD value of Positive Control must be greater than, or equal to 0.5 at 450 nm

Interpretation of results

The following is a guide to interpretation of anti-ET-1 antibody results. Each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered. Each laboratory must use known samples as further controls and establish their own anti-ET-1 Ab index cut-off values for positive/ negative results.

anti-ET-1 antibody index

Positive Result: If the anti-ET-1 Ab Index of sample is greater than 1.3, the sample is considered to be seropositive for anti-ET-1 antibody.

Equivocal Result: If the anti-ET-1 Ab Index of sample is between 0.8 – 1.3, the result is considered to be equivocal. Sample should be retested with another technique

Negative Result: If the anti-ET-1 Ab Index of sample is less than 0.8, the result is considered to be negative.

Specificity

The microplate is coated with highly purified Rat ET-1.

Reproducibility

- Inter-assay- <8.8%
- Intra-assay- <6.3%

Sample Dilution

Dilution of 1:50 is recommended. The user may need to determine the dilution factor in a preliminary experiment. If required, samples should be diluted in sample diluent buffer.

Scientific Background

Endothelins are peptides that constrict blood vessels and raise blood pressure. They are normally kept in balance by other mechanisms, but when they are over-expressed, they contribute to high blood pressure (hypertension) and heart disease.

Endothelins are 21-amino acid vasoconstricting peptides produced primarily in the endothelium having a key role in vascular homeostasis. Endothelins are implicated in vascular diseases of several organ systems, including the heart, general circulation and brain.

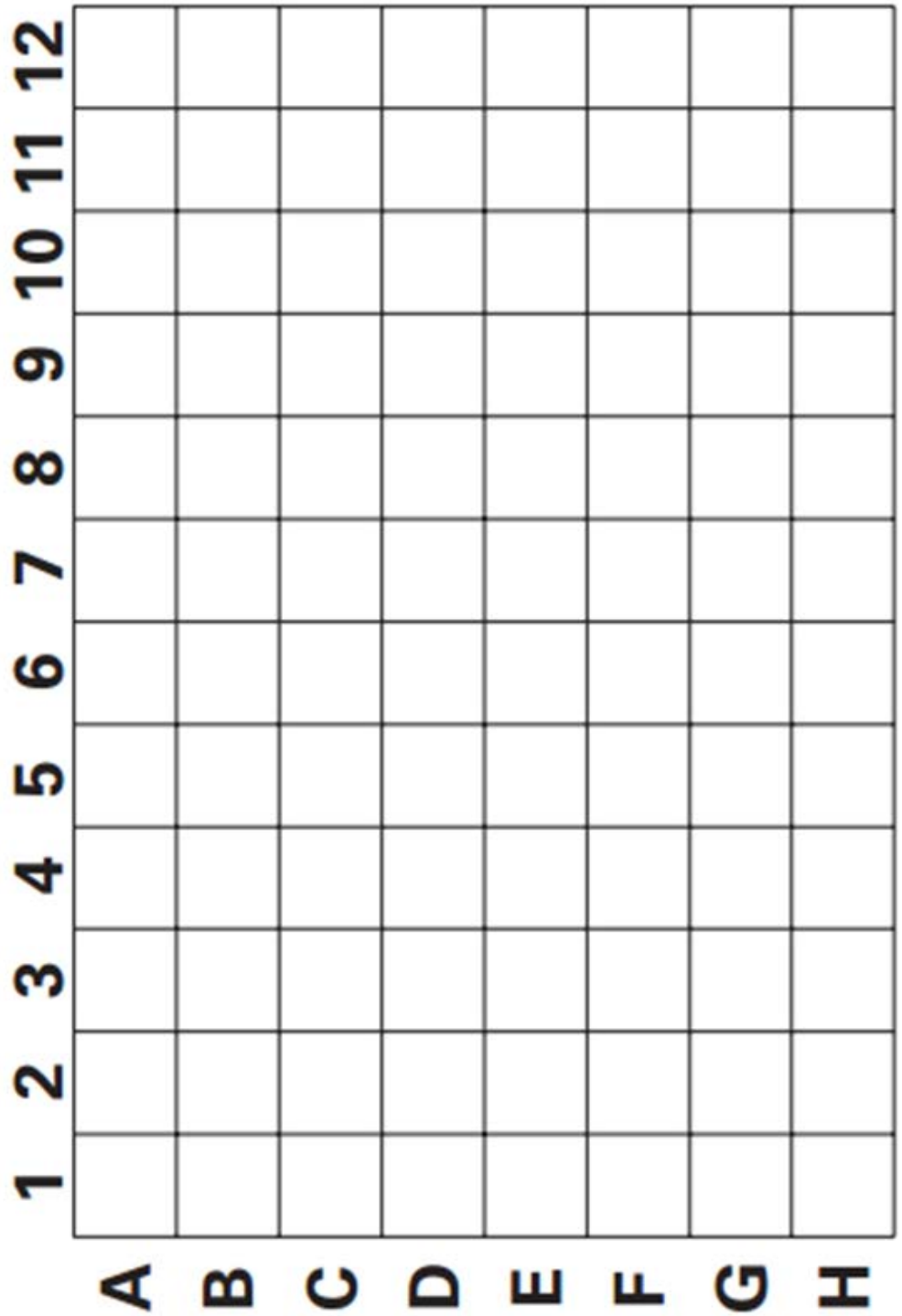
Endothelins are the most potent vasoconstrictors known.[5] In a healthy individual, a delicate balance between vasoconstriction and vasodilation is maintained by endothelin and other vasoconstrictors on the one hand and nitric oxide, prostacyclin and other vasodilators on the other.

Overproduction of endothelin in the lungs may cause pulmonary hypertension, which can sometimes be treated by the use of an endothelin receptor antagonist, such as bosentan, sitaxentan or ambrisentan. The latter drug selectively blocks endothelin A receptors, decreasing the vasoconstrictive actions and allowing for increased beneficial effects of endothelin B stimulation, such as nitric oxide production. The precise effects of endothelin B receptor activation depends on the type of cells involved.

For trouble shooting information please visit the following website:
http://www.novateinbio.com/en/content/15-tech-info *OR*

Email us at techsupport@novateinbio.com

Plate Layout



Troubleshooting Information

High Background

Probable Cause:	Solution/ Action
High incubation temperature:	Incubate at room temperature (25 °C) throughout the procedure
Insufficient washing of the plate:	Fill the wells with wash buffer and aspirate completely for the next wash Increase the number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, if available and check that all the channels are operating properly
Concentrated streptavidin-HRP	Streptavidin-HRP was not diluted properly Dilute the streptavidin-HRP as mentioned in the manual
Light exposure during substrate incubation	The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.
Stop solution not added	Color will continue to develop if stop solution is not added
Diluents came with the kit were not used	Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits
Contaminated solutions	Prepare fresh working solutions

Poor Standard Curve

Probable Cause:	Solution/ Action
Improper standard reconstitution:	Spin the vial briefly before opening Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature Do not store and reuse diluted standards
Curve fitting problem:	Log transform the values on both axes Use 4-PL/ 5-PL curve fitting programs
Incubation temperature/ time	Use the recommended standard incubation conditions
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.

No Signal

Probable Cause:	Solution/ Action
Omission of reagent(s):	Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual
Incorrect detection antibody was used:	Use the detection antibody came with the kit
Chromogen solutions were mixed improperly	Use the recommended procedure to prepare the TMB substrate
HRP inhibitor in sample/ buffers	Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.
Vigorous washing	If the washing is done manually, pipette the wash buffer gently.
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations
Improper plate reader settings	Check the wavelength and read the plate again

Erratic duplicate OD values

Probable Cause:	Solution/ Action
Insufficient washing of the plate	Fill the wells with wash buffer and aspirate completely for the next wash Increase number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, is available and check that all the channels are functioning properly
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.
Improper mixing of samples/ buffers	Mix the samples well before pipetting Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP
Contamination from other wells	Do not reuse the adhesive covers from previous assay setups Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well
Precipitates in the samples/ buffer	If precipitates are visible in wash buffer concentrate, keep it at 37 ^{o}C for 10-15 minutes until no precipitates are visible Centrifuge the samples to remove particulate matter
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations