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ab133030
Endothelin 1 ELISA Kit

For quantitative detection of Endothelin 1 in serum, plasma and culture fluids.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Endothelin 1 (ET-1) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Endothelin 1 in serum, plasma and culture fluids. ET-1 has identical amino acid sequence for Human, mouse, rat, cow, dog, pig, and rabbit.

Endothelin 1 specific antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and along with an HRP-conjugated I Endothelin 1 detection antibody and the microplate is then incubated at room temperature. After the removal of unbound proteins by washing, TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a colored product that changes after adding acidic stop solution. The density of coloration is directly proportional to the Endothelin 1 amount of sample captured in plate.

Endothelins (ET) were first identified as Endothelin - derived relaxation factors then again as an Endothelin - derived contracting factor. The predominant physical feature of ET's is the central helical core that is stabilized and includes two intra - chain disulfide bonds. This conformation is necessary for high affinity binding by the ETA receptor, but is not for binding by ETB. Translated as a pre - pro peptide and translocated into circulation as the pro form Big ET - 1, bioactive ET - 1 is released when Big ET - 1 is cleaved by Endothelin - converting enzymes at the site of action. Once released, ET - 1 is able to elicit different vaso - actions depending on which receptor is bound. The basal circulating level of ET - 1 is reported to be < 1 to 3 pg/mL but is known to be elevated in atrial and pulmonary hypertensions, atherosclerosis, congestive heart failure, cancer and variably related to lung diseases such as COPD and asthma.

2. Protocol Summary

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

Add standard or sample to each well used. Incubate wells.

Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate wells.

Aspirate and wash each well. Add Chromogen Substrate Solution to each well. Immediately begin recording the color development.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse monoclonal Endothelin 1 Microplate (12 x 8 wells)	96 wells	+4°C
Assay Buffer 17	100 mL	+4°C
Endothelin 1 Standard	250 µL	+4°C
20X Wash Buffer Concentrate	100 mL	+4°C
Endothelin 1 Antibody Concentrate	100 µL	+4°C
Antibody Diluent	10 mL	+4°C
TMB Substrate	10 mL	+4°C
Stop Solution 2	10 mL	+4°C
Plate Sealer	3 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- Eppendorf tubes.
- Microplate Shaker.
- Absorbent paper for blotting.
- Deionized or distilled water
- Acetic Acid
- Methanol
- Ethyl acetate
- Ammonium bicarbonate
- 200 mg C18 Sep-Pak column

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- Stop Solution is caustic; care should be taken in use.
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Wash Buffer

Dilute as much the 20X Wash Buffer as needed 1 in 20 with distilled water. Store the wash buffer at room temperature for up to three months or until the kit expiry day, whichever is sooner.

9.2 Endothelin 1 Antibody

Prepare the antibody by diluting 10 µL of the supplied antibody concentrate with 1 mL of antibody diluent for every mL of 1X needed. The diluted antibody must be used within 8 hours. Only prepare what is needed each day. Discard any unused, diluted antibody.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Diluted standards should be used within 30 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Allow the 1,000 pg/mL Endothelin 1 **Stock Standard** solution to equilibrate to room temperature.

10.2 Label nine tubes with numbers 1 – 9.

10.3 Add 250 µL of the Assay Buffer into tubes numbers 2 - 9.

10.4 Prepare a 100 pg/mL **Standard 1** by transferring 50 µL of the 1,000 pg/mL Stock Standard to 450 µL of the Assay Buffer into tube 1. Mix thoroughly and gently.

10.5 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 through 8.

10.8 **Standard 9** contains no protein and is the blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	50 µL Standard	450	1,000	100
2	250 µL Standard #1	250	100	50
3	250 µL Standard #2	250	50	25
4	250 µL Standard #3	250	25	12.5
5	250 µL Standard #4	250	12.5	6.25
6	250 µL Standard #5	250	6.25	3.13
7	250 µL Standard #6	250	3.13	1.56
8	-	250	1.56	0.78
9	-	250	-	0

11. Sample Preparation

- Culture fluids, serum, and plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay.
- Plasma samples should be drawn into chilled EDTA tubes (1mg/mL blood) containing Aprotinin (500 KIU/mL of blood). Centrifuge the blood at 1,600 x g for 15 minutes at 0°C. Transfer the plasma to a plastic tube and store at -70°C. Aliquot to avoid repeated freeze/thaw cycles.
- In humans, normal plasma levels of Endothelin-1 have been reported to be in the Range of 1-3 pg/mL. In certain disease states, levels may increase 3 fold or more.
- Samples with very low levels of ET-1 or with high levels of protein (e.g. serum and plasma), may require extraction for accurate measurement. Extraction of the sample should be carried out using a similar protocol to the one described below

    11.1.1 Add an equal volume of 20% acetic acid (AA) to the sample. Centrifuge at 3,000 x g for 10 minutes at 4°C to clarify; save the supernatant.
    11.1.2 Equilibrate a 200 mg C18 Sep - Pak column with one column reservoir volume (CV) 100% methanol (MeOH), followed by one CV water and one CV 10% MeOH.
    11.1.3 Apply the supernatant to the Sep - Pak column and wash with one CV 10% AA. Remove the excess AA by applying reduced pressure. Discard washes.
    11.1.4 Wash column with two CVs ethyl acetate and remove the excess by applying reduced pressure.
    11.1.5 Elute the sample slowly by applying 3 mL 100% MeOH/ 0.05 M ammonium bicarbonate (80/20 v/v). Collect the eluant in a plastic tube.
    11.1.6 Evaporate to dryness using a centrifugal concentrator under vacuum.
    11.1.7 If samples cannot be assayed immediately, store at -20°C.
    11.1.8 Reconstitute with at least 250 µL of the Assay Buffer and measure immediately.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL of standards and samples to the appropriate wells.

13.3 Seal the plate and Incubate for 1 hour at room temperature.

13.4 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 4 more times for a total of 5 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.5 Add 100 µL of Endothelin 1 antibody into each well.

13.6 Seal the plate. Incubate the plate at room temperature for 30 minutes.

13.7 Wash as described in step 13.4.

13.8 Add 100 µL of the TMB Substrate solution to every well. Incubate at room temperature for 30 minutes.

13.9 Add 100 µL Stop Solution into each well. The plate should be read immediately.

13.10 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

14. Calculations

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

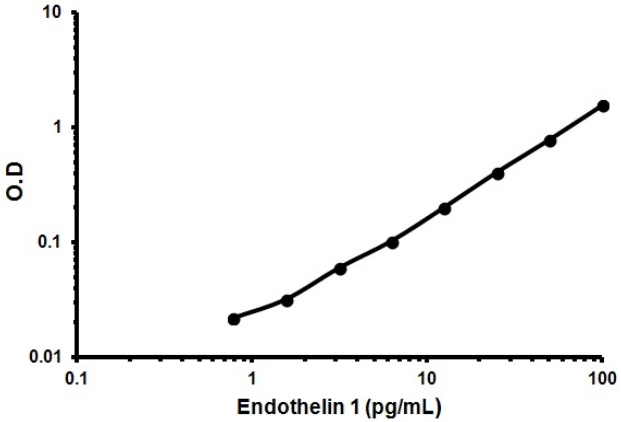
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

  Average Net OD = Average Bound OD – Average blank control OD

- Plot the average Net OD for each standard versus Endothelin 1 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean O.D. (-Blank)	Endothelin 1 Conc. (pg/mL)
Standard 1	1.563	100
Standard 2	0.786	50
Standard 3	0.409	25
Standard 4	0.202	12.5
Standard 5	0.103	6.25
Standard 6	0.060	3.13
Standard 7	0.032	1.56
Standard 8	0.022	0.78
Standard 9	0.007	0
Unknown 1	0.555	34.83
Unknown 2	0.027	1.19

Figure 1. Example of Endothelin 1 standard curve.

16. Typical Sample Values

PLASMA AND SERUM –

The following samples were tested for the presence of ET-1:

Sample Type	Number of Samples tested	Range (pg/mL)	Mean (pg/mL)
Human EDTA Plasma	6	1.1 – 2.4	1.8
Human Serum	6	1.2 – 2.5	1.8

N.B. Concentrations are corrected for extraction efficiency.

SENSITIVITY –

The sensitivity of the assay, defined as the concentration of ET-1 measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 0.41 pg/mL.

LINEARITY OF DILUTION –

A sample containing 80.25 pg/mL Endothelin 1 was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	-	80.25	-
1:2	40.12	37.62	93.8
1:4	20.06	19.08	95.1
1:8	10.03	9.97	99.4
1:16	5.02	5.12	102.0
1:32	2.51	2.56	102.0
1:64	1.25	1.49	119.2

PRECISION –

	Endothelin 1 (pg/mL)	Intra-Assay %CV
Low	1.1	8.8
Medium	2.3	8.9
High	35.9	6.7

	Endothelin 1(pg/mL)	Inter-Assay %CV
Low	1.2	15.6
Medium	2.5	5.9
High	35.1	8.3

17. Assay Specificity

CROSS REACTIVITY –

The cross reactivities for a number of related compounds were determined by diluting cross reactant in the assay buffer at a concentration of 10,000pg/mL.

ET-1	100 %
ET-2	21 %
ET-3	3.6 %
Human Big ET-1	<0.1 %
Rat Big ET-1	<0.1 %
Human Big ET-2	<0.1 %
Human Big ET-3 Amide	<0.1 %

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

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