ab285352 – Endothelin-1 ELISA Kit

For in vitro quantitative determination of Endothelin-1 in biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285352

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Mouse MAb to ET-1 pre-coated Microplate	1 unit	4°C
ET-1 Ab Concentrate	0.1 mL	4°C
Antibody Diluent	10 mL	4°C
Assay Buffer 17	100 mL	4°C
Wash Buffer Concentrate	100 mL	4°C
ET-1 Standard (1000 pg/ml)	0.25 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution 2 (1 N HCl in H20)	10 mL	4°C
Plate Sealer	3 units	4°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Absorbent paper
- Microplate shaker, centrifugal evaporator

Reagent Preparation

ET-1 Ab Concentrate: Prepare the antibody by diluting 10 µl of the supplied antibody concentrate with 1ml of antibody diluent for every ml of 1X needed. The diluted antibody must be used within 8 hrs. Prepare as per the assay requirement. Discard any unused, diluted antibody.

Wash Buffer: Prepare Wash Buffer by diluting 50 ml of the concentrate supplied with 950 ml of deionized water. This can be stored at room temperature until the kit expiration or for 3 months, whichever is earlier.

Sample Preparation

1. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.
2. Plasma samples should be drawn into chilled EDTA tubes (1mg/ml blood) containing Aprotinin (500 KIU/ml of blood).
3. Centrifuge the blood at 1,600 x g for 15 min. at 0°C. Transfer the plasma to a plastic tube and store at -70°C. Aliquot to avoid repeated freeze/thaw cycles. 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 here.
4. Add an equal volume of 20% acetic acid (AA) to the sample. Centrifuge at 3,000 x g for 10 min. at 4°C to clarify; save the supernatant. 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.
5. 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.
6. Wash column with two CVs ethyl acetate and remove the excess by applying reduced pressure.
7. 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.
8. Evaporate to dryness using a centrifugal concentrator under vacuum. If samples cannot be assayed immediately, store at -20°C. Reconstitute with at least 250 µl of the assay buffer and measure immediately.

Δ Note: *A minimum 1:4 dilution is required for unextracted serum and plasma samples to remove matrix interference in the assay. Culture fluids and extracted samples may be used without dilution. The optimal dilution for a specific experiment should be determined by the investigator.*

Assay Procedure

1. Place the desired no. of coated strips into the holder. Keep any unused wells with the desiccant back into the pouch, seal and store at 4°C.
2. Endothelin-1 Standard: Label eight 12 x 75 mm polypropylene tubes 1 through 8. Pipet 450 µl of Assay Buffer into tube 1. Pipet 250 µl of Assay Buffer into tubes 2 through 8. Add 50 µl of the 1000 pg/mL standard to tube 1. Vortex thoroughly. Add 250 µl of tube 1 to tube 2 and vortex thoroughly. Add 250 µl of tube 2 to tube 3 and vortex. Continue this for tubes 4 through 8. The concentration of Endothelin-1 in tubes 1 through 8 will be 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 pg/mL, respectively. Note: Diluted standards should be used within 30 min. of preparation.
3. Pipet 100 µl of Assay Buffer into S0 (0 pg/ml Standard) well(s).
4. Pipet 100 µl of standards 1-8 and samples into the appropriate wells.
5. Seal the plate. Incubate at room temperature for 1 hr.
6. Empty the contents of the wells and wash by adding 400 µl of 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 dry on a lint free paper towel to remove any remaining wash buffer.
7. Add 100 µl of diluted antibody into each well except the blank.
8. Seal the plate. Incubate at room temperature for 30 min.
9. Wash as above (step 5).
10. Pipet 100 µl of TMB substrate solution into each well. Incubate at room temperature for 30 min.
11. Pipet 100 µl of stop solution into each well.
12. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Calculations

Several options are available for the calculation of the concentration of Endothelin-1 in samples. We recommend that the data be handled by an immunoassay software package utilizing a four-parameter logistic curve-fitting program. Such software is often supplied by plate reader

manufacturers. Samples with concentrations outside of the standard curve range will need to be reanalyzed using a different dilution. If this sort of data reduction software is not readily available, the concentration of Endothelin-1 can be calculated as follows:

1. Calculate the average net OD bound for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample:

   Average Net OD = Average OD- Average Blank OD

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

Technical Support

For all technical or commercial enquiries please go to:

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