Endothelin-2 (1-31) Assay Kit - IBL

96 Well

Please read carefully this instruction prior you use this assay kit.

INSTRUCTIONS FOR USE

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

KIT COMPONENT

1	**Precoated plate:** (Anti-Endothelin-2$^{25\text{-}31}$ Rabbit IgG A.P.)	96Well x 1
2	**Labeled antibody:** HRP conjugated Anti-Endothelin-2 Rabbit IgG Fab' A.P)	10.5mL x 1
3	**Standard:** (Endothelin-2 (1-31))	0.5mL x 1
4	**EIA buffer**	30mL x 1
5	**Solution for labeled antibody**	12mL x 1
6	**Chromogen:** TMB solution	15mL x 1
7	**Stop solution**	12mL x 1
8	**Wash buffer conc.**	50mL x 1

MEASURING SAMPLES

Serum, EDTA-plasma, Cell culture supernatant and Tissue extract.

PRINCIPLE

This kit is a solid phase sandwich ELISA (Enzyme-linked Immunosorbent Assay). As a primary antibody is coated on a plate, samples and standard are added into the wells for 1st reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

OPERATING PRECATION

1 Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2 Test samples should be diluted with "4, EIA buffer" contained in this kit.
3 Duplicate measurement of test samples and standards is recommended.
4 Standard curve should run for each assay.
5 Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6 All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7 Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8 Fill the wash buffer each well, invert the plate and make sure the liquid is completely removed by shaking it off if you use a washing bottle. Repeat this washing process several times as instructed in order to avoid any insufficient washing process.
9 After remove the wash buffer, tapping the plate against a clean paper towel for completely removing the liquid from the wells and make sure the paper towel is not contact with inside of the wells in this process.
10 "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11 After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
12 Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".
13 Storage of HRP conjugated antibody is not recommended. However, if the HRP conjugates do not use at one time, please store it at below -20℃.

OPERATION MANUAL AND DOSAGES

1. Materials needed but not supplied.

Plate reader
Test tubes for dilution
Deionized water
Paper towel
Incubator (37°C±1°C)
Micropipette and tip
Measuring cylinder and beaker
Plate washer
Collecting container
(i.e. clean disposable test tube)
Refrigerator

2. Preparation

(1) Preparation of wash buffer

Dilute "8, Wash buffer conc." 40 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.

(2) Preparation of labeled antibody

Add 10.5mL of "5 Solution for labeled antibody" into "2, Labeled antibody" and leave it for 5 minutes and invert and mixed it well for completely dissolving the powder. This operation should be done immediately prior applying the labeled antibody into wells.

(3) Preparation of standard

Add 0.5 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 1000 pg/mL.
Prepare 7 test tubes for dilution of the standard and adding 230 µL of the EIA buffer into each tube.

Put 230 µL of 1000 pg/mL standard into the tube 500 pg/mL (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 250 pg/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 8 points of diluted standard between 500 pg/mL and 3.91 pg/mL.

Tube-1	500	pg/mL
Tube-2	250	pg/mL
Tube-3	125	pg/mL
Tube-4	62.5	pg/mL
Tube-5	31.25	pg/mL
Tube-6	15.63	pg/mL
Tube-7	7.81	pg/mL
Tube-8	3.91	pg/mL

(4) Preparation of test samples
Test sample should be diluted with "4, EIA buffer" as the need arises.
It is necessary to pre-extraction procedure by Sep-Pak C-18 column if you would like to apply serum, plasma or tissue samples. (see "Attention for sample handling" at the next page).

3 MEASUREMENT PROCEDURE

(1) Add test sample blank
Determine wells for test sample blank. Put 100µL each of "4, EIA buffer" into the wells.
(2) Add prepared test samples and standard
Put 100 µL prepared test samples and 100 µL prepared standard into appropriate wells.
(3) Incubation with plate lid (1st reaction).
(4) Washing
Wash the plate with the prepared wash buffer and remove all liquid.
(5) Add prepared labeled antibody
Put 100 µL prepared labeled antibody into the wells.
(6) Incubation with plate lid (2nd reaction).
(7) Washing
Wash the plate with the prepared wash buffer and remove all liquid completely.
(8) Add "6, Chromogen - TMB solution"
Put 100 µL the TMB solution into the wells.
(9) Incubation in dark
(10) Add "7, Stop solution"
Put 100 µL the Stop solution into the wells.
(11) Determination of optical density (O.D.)
Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, measure the both O.D. of standard and the test samples against a test sample blank.
Measurement wavelength: 450 nm. In case of 2 wavelengths:
Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

Table for measurement procedure

	Test samples	Standard	Test sample blank
Reagents	Test samples 100 µL	Diluted Standard 100 µL	EIA buffer 100 µL
1st reaction	Incubation for overnight at 2~8°C with plate lid.		
Washing	4 times (wash buffer more than 350 µL)		
Labeled antibody	100 µL	100 µL	100 µL
2nd reaction	Incubation for 30 minutes at 37°C with plate lid.		
Washing	5 times (wash buffer more than 350 µL)		
TMB solution	100 µL	100 µL	100 µL
Chromogenic reaction	Incubation for 30 minutes at R.T. (shielded).		
Stop solution	100 µL	100 µL	100 µL
Measuring O.D.	450 nm / 600～650 nm		

CALCULATION OF TEST RESULT

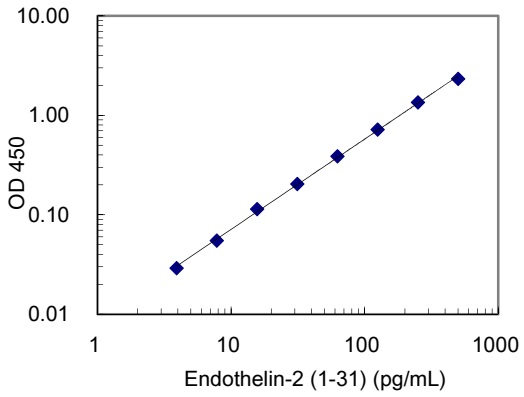
1 Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. quadratic regression of double logarithm conversion).
2 Read the concentration by applying the absorbance of the test samples on a standard curve.

Manufacturer: Immuno-Biological Laboratories Co., Ltd. URL: http://www.ibl-japan.co.jp E-mail: do-ibl@ibl-japan.co.jp

3 Calculate the concentration of the test samples by multiplying dilution ratio of test samples on the value.

Example of standard curve and measured value

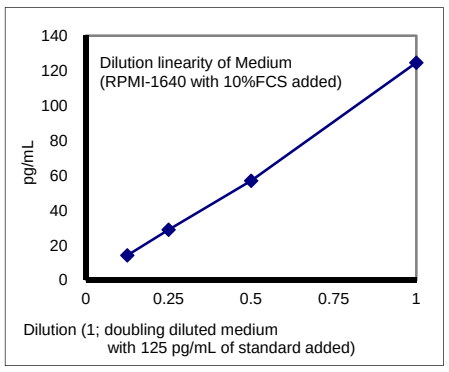
Standard (pg/mL)	O.D. (450nm)
500	2.325
250	1.350
125	0.720
62.5	0.387
31.25	0.204
15.63	0.114
7.81	0.055
3.91	0.029

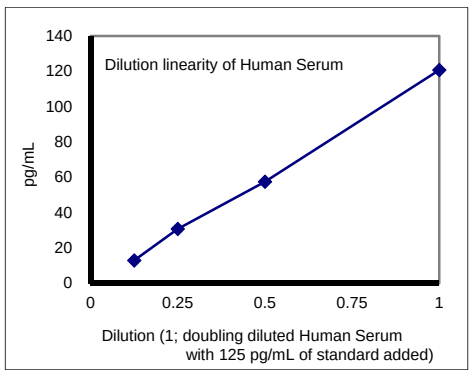


PERFORMANCE AND CHARACTERISTICS

1 Measurement range
3.91 ～ 500 pg/mL

2 Dilution linearity





3 Added recovery assay

Test samples	Theoretical value (pg/mL)	Measurement value (pg/mL)	%
Added 10%FCS Supplemented RPMI-1640	125	124	99.2
	62.5	60.8	97.3
	31.3	31.1	99.4
	15.6	14.6	93.6
Human Serum (Healthy subject) (x4)	125	117.3	93.8
	62.5	57.4	91.8
	31.3	31.2	99.7
	15.6	15.2	97.4

4 Intra-assay

Measurement value (pg/mL)	SD (pg/mL)	CV (%)	n
295.55	5.99	2.0	8
69.80	2.06	3.0	8
14.59	1.04	7.1	8

5 Inter-assay

Measurement value (pg/mL)	SD (pg/mL)	CV (%)	n
284.72	12.38	4.3	16
66.03	6.47	9.8	16
14.72	1.11	7.5	16

6 Specificity

Substance	Cross reactivity (%)
Endothelin-2 (1-31)	100
Endothelin-1 (1-31)	1.08
Endothelin-3 (1-31)	≦0.1
Endothelin-1 (1-21)	≦0.1
Endothelin-2 (1-21)	≦0.1
Endothelin-3 (1-21)	≦0.1
Big Endothelin-1	0.17
Big Endothelin-2	7.98
Big Endothelin-3	≦0.1
VIC (Mouse Endothelin-2)	≦0.1
Rat Big Endothelin-1	0.20

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1 Precaution for handling (Hazard prevention)

(1) Treat the components carefully and wash hands after handling it.

(2) “7, Stop solution” is a strong acid substance (1N Sulfuric acid). Therefore, it should be careful for the treatment and do not contact your skin and clothes with it. It also needs to pay attention to the disposal of it.

2 Precaution for intended use

(1) “2, Labeled antibody.” And “3, Standard” are lyophilized products. It should be careful to open this vial.

(2) Should be stored at 2~8°C.

(3) Precipitation can be seen in “4, EIA buffer” and “8, Wash buffer conc.”, however, it does not affect its performance.

(4) Do not mix or replace the reagents with the reagents from a different lot or kit.

(5) Do not use expired reagents.

3 Precaution for disposal

(1) Dispose used materials after rinsing them with large quantity of water.

Attention for sample handling:
This kit will allow a direct assay samples containing a low concentration of protein (e.g. cell culture media, urine and so on). However, extraction and concentration of Endothelin from samples will be required for samples containing a high concentration of protein (e.g. plasma, serum, tissue homogenates and so on). Extraction of test sample with Sep-Pak C-18 column is recommended as below:

1. Pre-treatment of Sep-Pak C-18 column (*1)
   a. Washing with 4mL of pure methanol.
   b. Washing 2 times with 2mL of distilled water.
   c. Washing 2 times with 2mL of 0.1% TFA solution

2. Pre-treatment of samples
   a. Plasma (serum) – Addition of 6mL of 10% CH_3COOH to 2mL of plasma with mixing
   b. Tissue sample
      (1) Addition of 1M CH_3COOH - 20mM HCl solution to tissue sample and homogenize.
      (2) After boiling for ten minutes, centrifuge at 10,000rpm for 10min and collecting a supernatant.

3. Extraction of sample
   a. Addition of treated sample to Sep-Pak C-18 column.
   b. Washing 3 times with 3mL of distilled water.
   c. Elution with 2mL of an appropriate solution (*2) and collection to vial

4. Measurement
   Collected sample in vial should be lyophilized and stored under frozen condition until measurement. Stored sample should be reconstituted with 0.1mL of an appropriate solution (*3) and added 0.2mL of “4, EIA buffer” and mixed. Confirm that the pH of sample is in a neutral range before measurement. There is a difference in recovery rate between samples. Please test added recovery assay in advance.

(*1) Product No. WAT023501, manufactured by Waters. (U.S.A.)
(*2) 0.1% Trifluoroacetic Acid (*4) plus 60% Acetonitrile in dH2O
(*3) No. 206-10731, manufactured by Wako Pure Chemical Industries Ltd. (Japan) is used in our protocol.
(*4) 0.1% Trifluoroacetic Acid in DMSO

STORAGE AND THE TERM OF VALIDITY

Storage Condition: 2~8°C
The expiry date is specified on the outer box.

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 17178

REFERENCES

Terui N, Suzuki H. CENTRAL NERVOUS SYSTEM AND BLOOD PRESSURE CONTROL 1992, Proceedings of The 7th Workshop on “Brain and Blood Pressure Copntrol” p.141-148

2. Wakisaka et al., Endothelin-1 kinetics in plasma urine, and blister fluid in burn patients. Annals of Plastic Surgery. 37, No.3, 305-309 1996

CONTACT DETAILS

Immuno-Biological Laboratories Co., Ltd.
1091-1 Naka, Fujioka-Shi, Gunma 375-0005
TEL：0274-22-2889
FAX：0274-23-6055