ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

ENDOTHELIN-1 (ET-1)
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K045-H1

Species Independent

Sample Types Validated:

Serum, Plasma and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

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BACKGROUND

Endothelin-1 (ET-1), a peptide of 21 amino acid residues, is a pleiotropic molecule known for its action as a potent vasoconstrictor[1]. ET-1 is one of a family of three proteins encoded by distinct genes that also includes Endothelin-2 (ET-2) and Endothelin-3 (ET-3)[2, 3]. ET-2 and ET-3 differ from ET-1 by 2 and 6 amino acids, respectively[1, 2]. All members of the Endothelin family contain two essential disulfide bridges and six conserved amino acid residues at the C-terminus. Human ET-1 is initially synthesized as a pre-pro-polypeptide of 212 amino acids[2, 4]. It is proteolytically cleaved by a signal peptidase to produce pro-ET-1 and further processed by a Furin-like protease to yield Big ET-1. Big ET-1 is then cleaved by the membrane-bound metalloprotease Endothelin-converting enzyme (ECE-1), producing the potent mature form, ET-1[5, 6]. The vascular endothelium is an abundant source of ET-1[3, 7]. It may also be expressed by leukocytes, smooth muscle cells, mesangial cells, cardiac myocytes, and astrocytes[8, 9]. ET-1 can be induced in endothelial cells by many factors including mechanical stimulation, various hormones, and pro-inflammatory cytokines. Production is inhibited by nitric oxide (NO), cyclic nucleotides, prostacyclin, and atrial natriuretic peptide (ANP)[10, 11].

ET-1 also stimulates cardiac contraction and the growth of cardiac myocytes, regulates the release of vasoactive substances, and stimulates smooth muscle cell mitogenesis. ET-1 may control inflammatory responses by promoting the adhesion and migration of neutrophils and stimulating the production of pro-inflammatory cytokines. It has also been implicated in cancer progression regulating the proliferation and migration of tumor cells and acting as a pro-angiogenic factor[13]. ET-1 has putative roles in other pathologies including septic shock, atherosclerosis, heart failure, renal insufficiency, pulmonary hypertension, and cerebrovascular conditions associated with subarachnoid hemorrhage[9].

1. Kawanabe, Y. and Nauli, SM., "Endothelin", Cell. Mol. Life Sci., 2011, 68:195-203
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3. Yanagisawa, M. et al., "A novel potent vasoconstrictor peptide produced by vascular endothelial cells," Nature, 1988, 332:411-415.
4. Inoue, A. et al., "The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression" J. Biol. Chem. 1989, 264:14954-14959.
5. D'Orleans-Juste, P. et al., "Synthesis and degradation of endothelin-1," Can. J. Physiol. Pharmacol., 2003, 81:503-510.
6. Xu, D. et al., "ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1" Cell, 1994, 78:473-485.
7. Yanagisawa, M., "The endothelin system. A new target for therapeutic intervention," Circulation, 1994, 89:1320-1322.
8. MacCumber, MW. et al., "Endothelin in brain: receptors, mitogenesis, and biosynthesis in glial cells.", Proc. Natl. Acad. Sci., 1990, 87:2359-2363.
9. Luscher, TF. and Barton, M., "Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs," Circulation, 2000, 102:2434-2440.
10. Boulanger, C. and Luscher, TF., "Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide," J. Clin. Invest., 1990, 85:587-590.
11. Stewart, D.J. et al., "Role of cyclic nucleotides in the regulation of endothelin-1 production by human endothelial cells," Am. J. Physiol., 1994, 266:H944-H950.
12. Sugden, P.H., J. Mol. Cell. Cardiol., 2003, 35:871.
13. Grant, K. et al., "Endothelin-1: a multifunctional molecule in cancer," Br. J. Cancer, 2003, 88:163-166.

ASSAY PRINCIPLE

The DetectX® Endothelin-1 (ET-1) kit is designed to quantitatively measure ET-1 present in a variety samples and tissue culture media. Please read the complete kit insert before performing this assay. An ET-1 standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture ET-1 present in the sample. After a 60 minute incubation, the plate is washed and a peroxidase conjugated ET-1 antibody is added. The plate is again incubated for 60 minutes and washed. Substrate is then added to the plate, which reacts with the bound ET-1 conjugated antibody. After a third incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the ET-1 in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Atrial Natriuretic Peptide (ANP) EIA Kits	K026-H1/H5
Cyclic AMP Direct EIA & CLIA Kits	K019-H1/H5, K019-C1/C5
Cyclic GMP Direct EIA & CLIA Kits	K020-H1/H5, K020-C1/C5
Hemoglobin Colorimetric Detection Kit	K013-H1
Nitric Oxide (NO) Detection Kit	K023-H1
Prostaglandin E_2 (PGE_2) Multi-Format EIA Kits	K051-H1/H5
Protein Kinase A (PKA) Activity Kit	K027-H1
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5

SUPPLIED COMPONENTS

Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with monoclonal antibody to human Endothelin-1.
One Plate — Catalog Number C161-1EA

Endothelin-1 Standard
Endothelin-1 at 10 ng/mL in a special stabilizing solution.
50 µL — Catalog Number C160-50UL

DetectX® Endothelin-1 Conjugate
An antibody to human Endothelin-1 labeled with peroxidase.
5 mL — Catalog Number C159-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL — Catalog Number X122-28ML

Extraction Solution
A solution used to extract Endothelin-1 from samples.
50 mL — Catalog Number X123-50ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL — Catalog Number X007-30ML

TMB Substrate
11 mL — Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.
5 mL — Catalog Number X020-5ML

Plate Sealer
2 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should then be stored at 4 °C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

A Speedvac or other centrifugal evaporator, or a manifold and inert gas supply such as nitrogen to evaporate extracted samples.

Polypropylene or glass test tubes.

Repeater pipet and disposable tips capable of dispensing 100 µL and 50 µL.

A microplate washer.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum, plasma, and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using. Due to the highly conserved nature of endothelin-1 it is expected that this kit will measure human, bovine, porcine, dog, rat and mouse ET-1. The end user should test this kit for application in their samples.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples must be extracted with the provided Extraction Solution, or with a solid phase C18 column extraction protocol (see Peptide/Protein Extraction Protocol at www.arborassays.com/resources/#protocols) prior to running in the kit.

Protocol Using Extraction Solution:

1. Mix 1 part sample with 1.5 parts of Extraction Solution.
2. Vortex and then mix at room temperature for 90 minutes.
3. Centrifuge for 20 minutes at 4°C at 1660 x g.
4. Transfer supernatant to a clean tube.
5. Speedvac supernatant to dryness at 37°C.
6. Reconstitute sample with 150 µL of Assay Buffer.

Tissue Culture Media Samples

TCM samples should be diluted ≥ 1:20 in Assay Buffer and read off the standard curve generated in Assay Buffer.

Any samples with concentrations outside the standard curve range should be diluted further with Assay Buffer, as appropriate, to obtain readings within the standard curve range.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

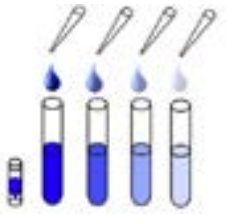
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Standard Preparation

Label test tubes as #1 through #8. Pipet 990 µL of Assay Buffer into tubes #1. Pipet 150 µL of Assay Buffer into tubes #2 to #8. The ET-1 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the 10 ng/mL ET-1 standard to tube #1 and vortex completely. Take 150 µL of the ET-1 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of ET-1 in the tubes #1 through #8 will be 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer Volume (µL)	**990**	150	150	150	150	150	150	150
Addition	**Stock**	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Volume of Addition (µL)	**10**	150	150	150	150	150	150	150
Final Conc (pg/mL)	**100**	50	25	12.5	6.25	3.125	1.563	0.781

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine ET-1 concentrations.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells.
3. Cover the plate with the plate sealer and incubate at room temperature for 60 minutes.
4. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
5. Add 50 µL of the DetectX® Endothelin-1 Conjugate to each well, using a repeater pipet.
6. Cover the plate with the plate sealer and incubate at room temperature for 60 minutes.
7. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
9. Incubate the plate at room temperature for 30 minutes.
10. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
11. Read the optical density generated from each well at 450 nm.
12. Use the plate reader's built-in 4PLC software capabilities to calculate Endothelin-1 concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-endothelin-1-(et-1)-eia-kit.assay

MyAssays

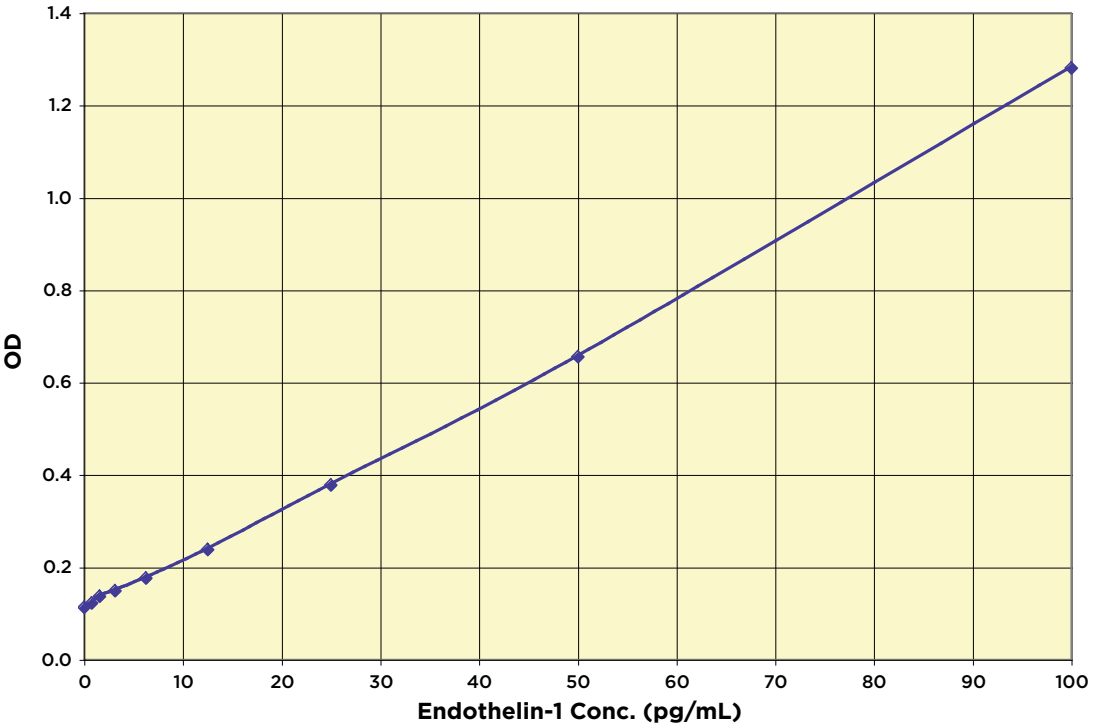
TYPICAL DATA

Sample	Mean OD	Endothelin-1 Conc. (pg/mL)
Standard 1	1.281	100
Standard 2	0.656	50
Standard 3	0.378	25
Standard 4	0.238	12.5
Standard 5	0.177	6.25
Standard 6	0.150	3.125
Standard 7	0.138	1.563
Standard 8	0.123	0.781
Zero	0.114	0
Sample 1	0.632	44.74
Sample 2	0.348	22.79

Always run your own standard curve for calculation of results. Do not use this data.

ARBOR ASSAYS®

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #8. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.579 pg/mL.

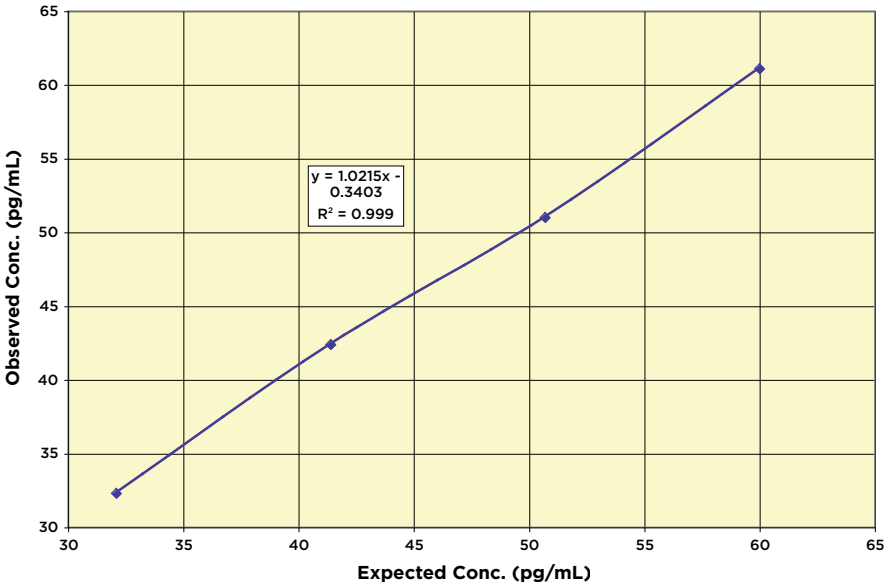
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration control sample.

Limit of Detection was determined as 0.807 pg/mL.

Linearity

Linearity was determined by taking two diluted samples, one with a low diluted ET-1 level of 22.8 pg/mL and one with a higher diluted level of 69.3 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	60.0	61.1	101.8%
60%	40%	50.7	51	100.6%
40%	60%	41.4	42.4	102.4%
20%	80%	32.1	32.3	100.6%
			Mean Recovery	**101.4%**



Intra Assay Precision

Three samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated ET-1 concentrations were:

Sample	Endothelin-1 Conc. (pg/mL)	%CV
1	81.7	3.7
2	54.0	4.1
3	28.0	3.3

Inter Assay Precision

Three samples were diluted with Assay Buffer and run in duplicates in eighteen assays run over multiple days by three operators. The mean and precision of the calculated ET-1 concentrations were:

Sample	Endothelin-1 Conc. (pg/mL)	%CV
1	73.9	5.3
2	49.0	4.6
3	25.9	6.0

SAMPLE VALUES

A number of human serum, plasma and urine samples were tested in the kit. Normal plasma samples extracted using the Extraction Solution provided in the kit gave ET-1 concentrations that ranged from 0.545 to 3.24 pg/mL with an average value of 1.13 pg/mL. Several EDTA plasma samples from patients suffering from heart disease ranged from 6.8 to almost 12 pg/mL. Several normal serum samples gave ET-1 concentrations that ranged from 3.1 to 4.8 pg/mL with an average value of 4.06 pg/mL

Human urine samples diluted 1:2 to 1:6 in Assay Buffer read between 1.9 and 2.8 pg/mL. One urine sample from a patient with a renal condition read at 4.56 pg/mL. After correction for creatinine using our Urinary Creatinine Detection kit, K002-H1, this sample read at 3.32 pg/mg creatinine.

CROSS REACTIVITY

The following cross reactants were tested in the assay and cross reactivity calculated within the standard curve.

Steroid	Cross Reactivity (%)
Endothelin-1 (human, bovine, porcine, dog, rat, mouse)	100%
Endothelin-3 (human, bovine, porcine, dog, rat, mouse)	6.8%
Big Et-1 (human)	< 0.04%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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FOR SERIOUS SCIENCE

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