Endothelin-1 (ET-1) ELISA Kit

Catalog Number EIAET1 (96 tests)

Rev 2.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Endothelin-1 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of endothelin-1 in serum, EDTA and heparin plasma, urine and tissue culture media. Characterization of this ELISA kit was done primarily on human samples, but cross-reactivity with bovine, porcine, dog, rat, and mouse samples was observed.

Endothelin-1 (ET-1) is a pleiotropic molecule of 21 amino acid residues involved in cardiac and vascular function, and inflammatory responses. It is highly expressed in the vascular endothelium, and also produced by leukocytes, smooth muscle cells, mesangial cells, cardiac myocytes, and astrocytes.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Endothelin-1 Standard; 10 ng/mL endothelin-1	50 µL
Assay Buffer Concentrate (5X)	28 mL
Endothelin-1 Antibody Coated Wells, 96-well strip-well plate	1 plate
Endothelin-1 Conjugate	5 mL
Extraction Solution	50 mL
Wash Buffer Concentrate (20X)	30 mL
Tetramethylbenzidine (TMB) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL
Plate Sealer	2

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare serum and plasma samples

Serum and plasma samples must be extracted with Extraction Solution or with a solid phase C18 column extraction protocol (see protocol on the product page at **thermofisher.com**) prior to running the assay.

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 at 1,660 × *g* for 20 minutes at 4°C.
4. Dry the sample at 37°C in a Speedvac™ vacuum concentrator.
5. Reconstitute the dried sample with 150 µL of 1X Assay Buffer.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

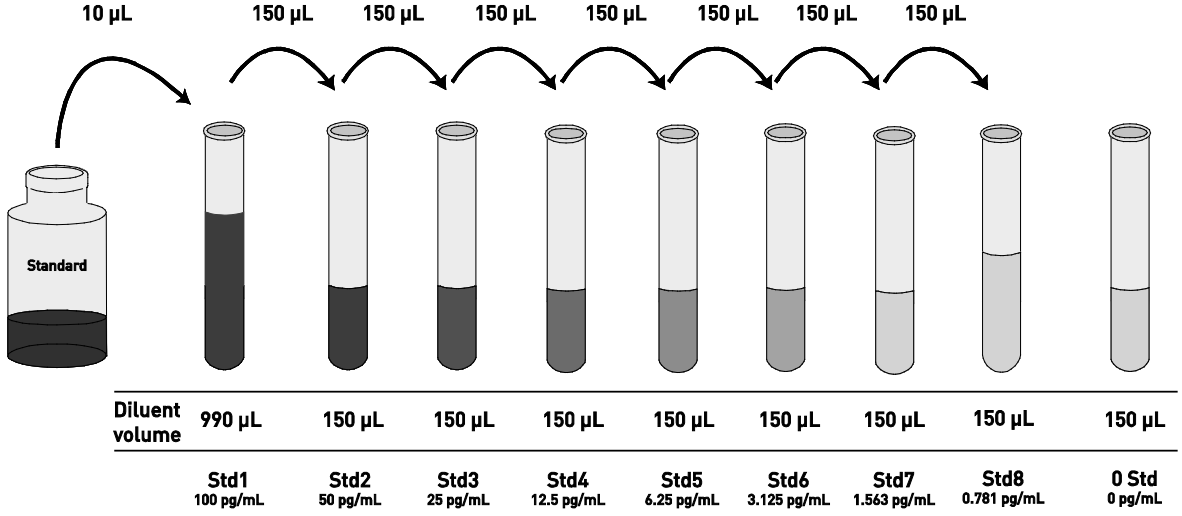
Use all samples within 2 hours of dilution.

Dilute **tissue culture media** samples ≥1:20 with 1X Assay Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 10 µL Endothelin-1 Standard to one tube containing 990 µL 1X Assay Buffer and label as 100 pg/mL ET-1.
   Note: The standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.
3. Add 150 µL Standard Diluent Buffer to each of 8 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, and 0 pg/mL ET-1.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.

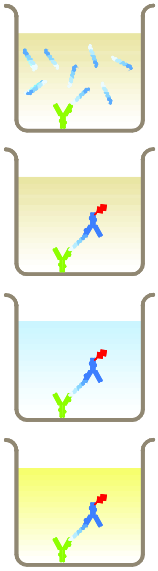


Perform ELISA (Total assay time: 2.5 hours)

IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



Bind antigen

a. Add 50 µL of standards or samples (see "Pre-dilute samples" on page 2) to the appropriate wells.
b. Cover the plate with plate sealer and incubate for 60 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add detection antibody

a. Add 50 µL Endothelin-1 Conjugate into each well.
b. Incubate for 60 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature.
Note: TMB should not touch aluminum foil or other metals.

Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals greater than that of the highest standard in 1X Assay Buffer or the appropriate tissue culture medium and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data was obtained for the various standards over the range of 0–100 pg/mL endothelin-1.

Standard Endothelin-1 (pg/mL)	Optical Density (450 nm)
100	1.281
50	0.656
25	0.378
12.5	0.238
6.25	0.177
3.125	0.150
1.563	0.138
0.781	0.123
0	0.114

Inter-assay precision

Samples were independently run three times in eighteen assays in duplicate to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	73.9	49.0	25.9
%CV	5.3	4.6	6.0

CV = Coefficient of Variation

Intra-assay precision

Samples of known endothelin-1 concentration were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	81.7	54.0	28.0
%CV	3.7	4.1	3.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

A number of human serum samples, plasma samples (extracted with Extraction Solution), and urine samples (diluted 1:2 to 1:6 in 1X Assay Buffer) were tested with the kit.

Sample	Range (pg/mL)	Average (pg/mL)
Plasma	0.6–3.2	1.1
Serum	3.1–4.8	4.1
Urine	1.9–2.8	—

Linearity of dilution

Linearity was determined by assaying high and low concentration samples (high sample 69.3 pg/mL of ET-1; low sample 22.8 pg/mL of ET-1), mixed in the ratios shown in the following table.

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%**

Specificity

Endothelin-1 is highly conserved, and it is expected that the assay can measure endothelin-1 from a number of different species. Each investigator should test the kit for application with their samples.

The following samples were tested using the assay and cross-reactivity calculated within the standard curve.

Sample	% 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

Sensitivity

The analytical sensitivity of the assay is 0.579 pg/mL endothelin-1. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288