# Endothelin ELISA Kit

Item No. 583151

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# GENERAL INFORMATION

## Materials Supplied

| Item Number | Item | Quantity/Size | Storage Temperature |
|---|---|---|---|
| 483152 | Anti-Endothelin ELISA Plate | 1 plate | -20°C |
| 483150 | Endothelin AChE-Fab' Conjugate | 1 vial/100 dtn | -20°C |
| 483154 | Endothelin ELISA Standard | 1 vial | -20°C |
| 400060 | ELISA Buffer Concentrate (10X) | 2 vials/10 ml | RT |
| 400062 | Wash Buffer Concentrate (400X) | 1 vial/5 ml | RT |
| 400035 | Polysorbate 20 | 1 vial/3 ml | RT |
| 400012 | 96-Well Cover Sheet | 1 cover | RT |
| 400050 | Ellman's Reagent | 3 vials/100 dtn | -20°C |
| 400100 | Human Plasma | 1 vial/100 dtn | -20°C |
| 400110 | Non-specific Mouse Serum | 1 vial/100 dtn | -20°C |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's AChE ELISA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

## If You Have Problems

**Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Email:** techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 14).

# INTRODUCTION

## Background

The endothelin (ET) peptide family consists of three isoforms, ET-1 (corresponding to the initially isolated and most predominant isoform), ET-2, and ET-3, each the product of separate genes.[1,2] Each isopeptide is 21 amino acids in length, with two intra-chain disulfide bonds. ET-2 and ET-3 exhibit 2 and 6 different amino acids, respectively, compared to ET-1 (see Figure 1, on page 7). ET-1 was originally isolated from the supernatant of cultured porcine aortic endothelial cells and is one of the most potent vasoconstrictors currently known.[3] Human ET-1 is derived from a 212 amino acid precursor protein (preproendothelin) which is cleaved by a neutral endopeptidase to form a 38 amino acid pro-ET-1 or "big endothelin".[1] Big endothelin is essentially devoid of biological acitivity. Endothelin-converting-enzyme-1 (ECE-1) converts big endothelin to the mature endothelin peptide. In addition to being a potent vasoconstrictor, ET-1 displays other pharmacological actions including bronchoconstriction and regulation of kidney function.[1,3,4] All three endothelins act through specific G protein-coupled receptors, $ET_A$ and $ET_B$, to activate phospholipases resulting in elevation of inositol phosphates, diacylglycerol, eicosanoids, and calcium.[5-7] ET-2 displays similar pharmacology to ET-1, whereas ET-3 is a weaker vasoconstrictor but more potent inhibitor of platelet aggregation.[1,6]

Normal plasma levels of ET-1 are very low (<1 pg/ml). However, elevated levels of ET have been detected in patients in cardiogenic shock, on chronic dialysis, and with pulmonary hypertension.[7] Elevated levels have also been detected in cases of hyponatremia, uremia, myocardial infarction, and several other medical disorders.[8-11]

## About This Assay

Cayman's Endothelin Assay is an immunometric (*i.e.*, sandwich) ELISA that permits endothelin measurements within the range of 7.8-250 pg/ml, typically with a limit of detection of 7.8 pg/ml. Inter- and intra-assay CVs of less than 10% may be achieved at most concentrations. This assay offers sensitive analysis of endothelin in serum, plasma, urine, or cell culture media.

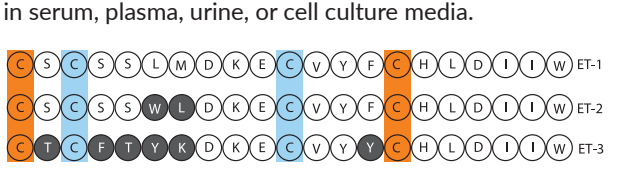


**Figure 1. Endothelin primary structure**

## Description of AChE Immunometric ELISAs[12]

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microtiter plate supplied with the kit has been coated with a monoclonal antibody specific for endothelin (endothelin capture antibody). This antibody will bind any ET-1, ET-2, or ET-3 introduced into the well. An acetylcholinesterase:Fab' Conjugate (AChE:Fab'), which binds selectively to a different epitope on the endothelin molecule, is also added to the well. When endothelin (standard or sample) is added to the well, the two antibodies form a 'sandwich' by binding on opposite sides of the endothelin molecule. The 'sandwiches' are immobilized on the plate so the excess reagents may be washed away. The concentration of the analyte is then determined by measuring the enzymatic activity of the AChE by adding Ellman's Reagent (which contains the substrate for AChE) to each well. The product of the AChE-catalyzed reaction has a distinct yellow color which absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound Conjugate which in turn is proportional to the concentration of the endothelin.

$$\text{Absorbance} \propto [\text{AChE:Fab' Conjugate}] \propto [\text{Endothelin}]$$

A schematic description of the assay is given in Figure 2 on page 9.

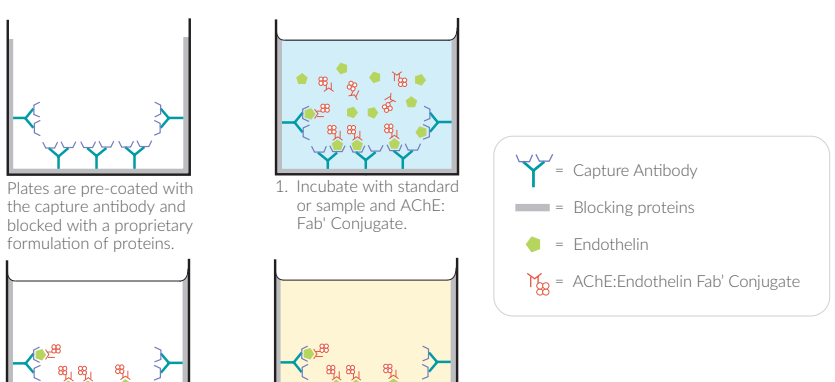


**Figure 2. Schematic of the AChE ELISA**

## Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *E. electricus*, contains an avid AChE capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover (64,000 $s^{-1}$) for the hydrolysis of acetylthiocholine.

A molecule of acetylcholinesterase covalently attached to an analyte-specific antibody serves as the conjugate in AChE enzyme immunometric assays. Quantification of the conjugate is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3, page 11). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm (ε = 13,600).

AChE has several advantages over other enzymes commonly used for enzyme immunoasays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows redevelopment of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.

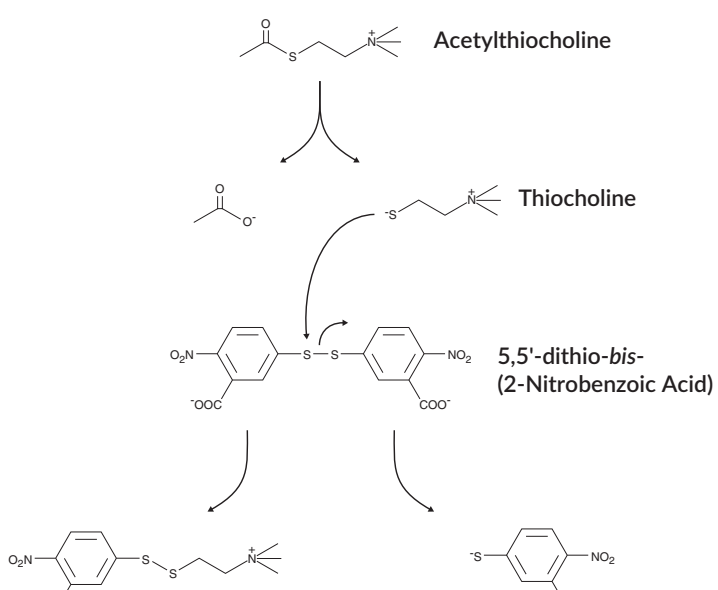


**Figure 3. Reaction catalyzed by acetylcholinesterase**

## Definition of Key Terms

**Blank:** background absorbance caused by Ellman's Reagent. Even freshly prepared Ellman's Reagent has some measurable absorbance, approximately 0.1 Absorbance Units (A.U.). The blank absorbance should be subtracted from the absorbance readings of all the other wells.

**Standard Curve:** a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

**Sample Matrix Blank (SMB):** in order to accurately assay unpurified samples, the standards must be present in the same biological fluid ('matrix') as the samples. One must obtain a supply of this 'matrix' (plasma, synovial fluid, cell culture medium, etc.) which does not contain endothelin; this is the Sample Matrix Blank (SMB). The SMB is used as the diluent for the standard curve. *NOTE: We supply a Human Plasma SMB as part of this kit. If your samples are in any other matrix, sufficient quantities of this matrix must be obtained to use as the diluent for your standard curve. If you do not have access to an appropriate SMB, you may eliminate the need for an SMB by purifying samples (see page 15). Note that purification can improve the sensitivity of this assay.*

**Dtn:** determination, where one dtn is the amount of reagent used per well.

**Cross Reactivity:** numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% $B/B_0$) value of the tested molecule to the mid-point (50% $B/B_0$) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[ \frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$



# PRE-ASSAY PREPARATION

*NOTE: Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA. UltraPure water may be purchased from Cayman (Item No. 400000).*

## Buffer Preparation

*Store all buffers at 4°C; they will be stable for about two months.*

1. **ELISA Buffer Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. Add 0.1 ml of Polysorbate 20 and mix thoroughly.

*NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035).

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer).

*NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

## Sample Preparation

In general, samples can be assayed with no prior purification. Some sample matrices inhibit binding of the antibodies used in this kit, so it may be necessary to use higher standard concentrations (or purify the samples) for a given SMB. For instance, plasma samples must be >50 pg/ml to be assayed accurately. Normal levels of endothelin-1 in human plasma are below the detection limit of this kit; therefore, purification and concentration of the sample is necessary for accurate measurements (see sample purification below). The concentrations of the standards must be adjusted to account for different SMBs (see page 18). If human plasma or synovial fluid is to be tested, one must add the non-specific mouse immunoglobulin (supplied in the kit) to each sample and each point of the standard curve. This will compensate for the effects of human anti-mouse IgG which may be present in the samples. Samples of synovial fluid from patients with rheumatoid arthritis often contain anti-mouse IgM (rheumatoid factors) which can cause erroneously high values. The addition of both the non-specific mouse serum and dithiothreitol (DTT) will alleviate this problem.[12] Remember, the standard curve wells must contain the same 'matrix' (including mouse serum and DTT) as the sample wells.

**General Precautions**

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.



## Sample Purification

Purification of the sample will eliminate uncertainty about the sample matrix; purified samples can be re-suspended in pure ELISA Buffer and quantified against standards that are also diluted in ELISA Buffer. Before deciding to purify your samples, be certain to consider the added labor involved and potential losses and error in recovery of endothelin. Recovery can be monitored by spiking samples with radioactive endothelin. Samples containing low concentrations of endothelin (0-50 pg/ml) should be purified and the standard curve prepared in ELISA Buffer rather than in the SMB.

### Testing for Interference

Plasma, serum, as well as other heterogeneous mixtures such as CSF often contain contaminants which can interfere in the assay. It is best to check for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between 10 and 250 pg/ml. If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated endothelin concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

### SPE (C-18) Purification Protocol

The following protocol, on pages 16-17, is a suggestion only. You may choose a different protocol based on your own requirements, sample type, and expertise. If desired, recovery may be tracked by spiking samples with radiolabeled endothelin and follow the spiked-sample recovery calculation on page 27. Otherwise, omit steps 2 and 10, on page 17.

**Materials Needed**

1. Radiolabeled endothelin (optional)
2. UltraPure water, methanol, 4% acetic acid in water, and 4% acetic acid in 86% ethanol
3. 500 mg C-18 solid phase extraction (SPE) columns (Item No. 400020)

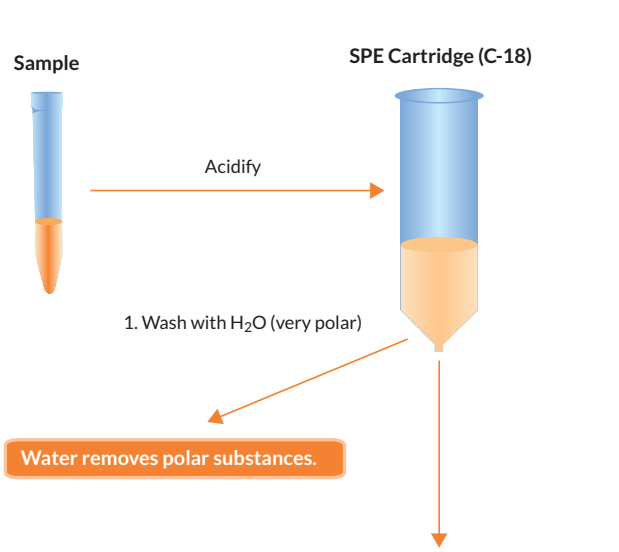


**Figure 4. Schematic of Endothelin Purification by SPE (C-18)**

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If your samples need to be concentrated, a larger volume should be used (*e.g.*, a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, *etc.*).
2. Add 10,000 cpm of radiolabeled endothelin.
3. Activate a 6 ml SPE Cartridge (C-18) (Item No. 400020) by rinsing with 5 ml methanol and then with 5 ml UltraPure water. *NOTE: Do not allow the SPE cartridge to dry.*
4. Dilute the sample 1:4 with 4% acetic acid in water (*i.e.*, dilute 1 ml of sample with 3 ml of 4% acetic acid).
5. Pass the sample slowly (approximately 1 ml/min) through the SPE cartridge using gravity flow.
6. Wash the column with 3-5 ml of UltraPure water.
7. Elute the endothelin using 2 x 1 ml applications of elution solution (prepared by mixing 10 ml water, 4 ml glacial acetic acid, and 86 ml of 100% ethanol).
8. Dry the sample by vacuum centrifugation or using a gentle stream of nitrogen. It is imperative that all the solvent be removed as even trace quantities can affect the ELISA.
9. Resuspend the sample in 500 µl of ELISA Buffer and vortex.
10. Use 50 µl of sample for scintillation counting to determine recovery. *NOTE: Samples can effectivly be concentrated by resuspending the sample in less volume than originally used for purification. The calculations on pages 26-27 will account for this volume change in the sample.*

# ASSAY PROTOCOL

## Preparation of Assay-Specific Reagents

1. **Sample Matrix Blank - Human Plasma**

   The SMB (Item No. 400100) provided with this kit is human plasma containing ≤1.5 pg/ml endothelin. If this matches your samples, reconstitute the 100 dtn vial with 5 ml of UltraPure water. Store this solution at 4˚C; it will be stable for approximately two weeks. If your SMB is not human plasma, you must obtain an endothelin-free SMB that matches your samples.

2. **Endothelin ELISA Standard**

   Reconstitute the Endothelin ELISA Standard (Item No. 483154) with 1 ml of ELISA Buffer. The concentration of this solution (the bulk standard) will be 5 ng/ml. Store this solution at 4°C; it will be stable for approximately two weeks. We have included enough endothelin to run five standard curves. This surplus should accommodate any experimental design.

   - **Plasma samples:** Obtain eight clean test tubes and number them #1 through #8. Aliquot 200 µl from the bulk standard into tube #1 and dilute to 1.0 ml with SMB and vortex briefly. The endothelin concentration of this tube is now 1,000 pg/ml and is the highest point on the standard curve. Next add 500 µl of SMB to tubes #2-8. Aliquot 500 µl from tube #1 into tube #2 and vortex briefly. The concentration of tube #2 will be 500 pg/ml. Add 500 µl from tube #2 to tube #3 and vortex briefly. The endothelin concentration of tube #3 will now be 250 pg/ml. Continue this procedure until tube #7 has been prepared (it will be 15.6 pg/ml). Do not add any endothelin to tube #8. This tube is the zero-point tube, the lowest point on the standard curve
   - **Purified Samples:** Follow the same steps above but start by diluting 50 µl of bulk standard to 1.0 ml with ELISA Buffer, making the tube #1 concentration 250 pg/ml. Be certain to make all dilutions with ELISA Buffer rather than SMB. Your standard curve will run from 250 pg/ml to 3.9 pg/ml, and your last point will be the 0 pg/ml point.
   - **Other Samples:** Dilute the standards using your endothelin-free SMB. The working range of standards must be determined prior to analyzing any samples. We recommend starting the curve at 500 pg/ml for the first attempt.

3. **Non-specific Mouse Serum**

   The mouse serum (Item No. 400110) supplied with this kit is to be used when analyzing unpurified plasma, serum, synovial fluid or any other sample that contains heterophilic antibodies.[15] Reconstitute the mouse serum with 2.5 ml (100 dtn vial) and store at 4˚C. It will be stable for approximately two weeks. A 25 µl aliquot of the mouse serum should be added to each 500 µl aliquot of sample or standard prior to addition to the well. Remember, you must also add the mouse serum to each point of the standard cure (25 µl of mouse serum per 500 µl of standard) to ensure a uniform SMB.

4. **Dithiothreitol (Not included in this kit)**

   If you suspect your samples contain anti-mouse IgM (rheumatoid factors), add 50 µl of 100 mM DTT to a 500 µl aliquot of each sample. Remember, you must also add the DTT to each point of the standard curve (50 µl of DTT per 500 µl of standard) to ensure a uniform SMB. *NOTE: This is a comparative assay. Although the addition of mouse serum and DTT will change the concentration of your samples and standards, the change will be proportional throughout the assay.*

5. **Acetylcholinesterase:Endothelin Fab' Conjugate**

   Reconstitute with 10 ml ELISA Buffer (Item No. 400060). Store the reconstituted conjugate at 4˚C and use within four weeks.

## Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 5 below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see **Analysis**, page 26, for more details). We suggest you record the contents of each well on the template sheet provided (see page 34).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | S1 | S1 | 1 | 1 | 1 | 9 | 9 | 9 | 17 | 17 | 17 | 25 |
| B | S2 | S2 | 2 | 2 | 2 | 10 | 10 | 10 | 18 | 18 | 18 | 25 |
| C | S3 | S3 | 3 | 3 | 3 | 11 | 11 | 11 | 19 | 19 | 19 | 25 |
| D | S4 | S4 | 4 | 4 | 4 | 12 | 12 | 12 | 20 | 20 | 20 | 26 |
| E | S5 | S5 | 5 | 5 | 5 | 13 | 13 | 13 | 21 | 21 | 21 | 26 |
| F | S6 | S6 | 6 | 6 | 6 | 14 | 14 | 14 | 22 | 22 | 22 | 26 |
| G | S7 | S7 | 7 | 7 | 7 | 15 | 15 | 15 | 23 | 23 | 23 | Blk |
| H | S8 | S8 | 8 | 8 | 8 | 16 | 16 | 16 | 24 | 24 | 24 | Blk |

Blk - Blank
S1-S8 - Standards 1-8
1-26 - Samples

**Figure 5. Sample plate format**

## Performing the Assay

**Pipetting Hints**

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### Addition of the Reagents

1. **Endothelin ELISA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

2. **Samples**

   Add 100 µl of sample per well. We recommend that each sample be assayed in triplicate, or at the very minimum in duplicate.

3. **Endothelin AChE Fab' Conjugate**

   Add 100 µl to each well *except* the Blk wells.

## Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate overnight at 4°C.

## Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use with 20 ml of ultrapure water. *NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.*
2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well.
4. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark.
5. The plates can be checked periodically over the next few hours. Once the S1 wells seem visibly yellow (0.3 A.U., ~20-30 minutes) it will be possible to determine the concentrations of the relatively concentrated samples. Longer development times will be necessary to obtain an accurate plot of the lower range of the standard curve and statistically significant values for sample concentrations near the detection limit of the assay (~7.8 pg/ml).

*NOTE: In general an immunometric assay will require longer development times to obtain an accurate plot for the lower range of the standard curve and statistically significant values for sample concentrations near the detection limit of the assay. This is demonstrated by the representative standard curves at various development times in Figure 6 (see page 24).*

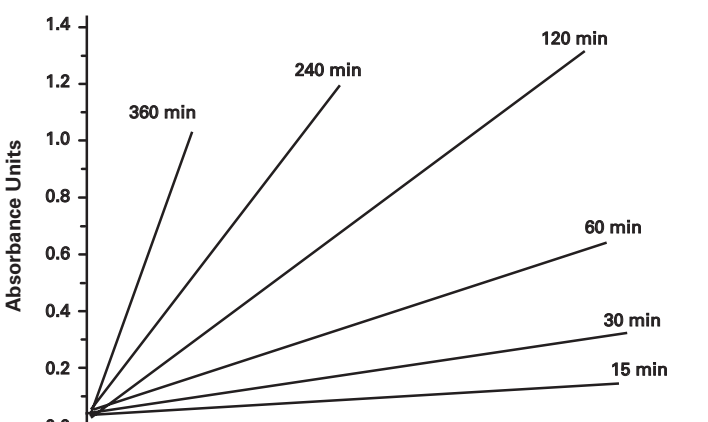


**Figure 6. Theoretical standard curve at various development times**

## Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*
3. Read the plate at a wavelength between 405 and 420 nm. If the absorbance of the wells exceeds 1.5, wash the plate, add fresh Ellman's Reagent, and let it develop again.

# ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program may be used. We recommend using a second order polynomial (quadratic) fit since the absorbance at the standard concentrations is slightly non-linear. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

## Calculations

### Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

1. If your plate reader has not already done so, subtract the average absorbance of the blank wells from the absorbance readings for the rest of the plate.
2. Calculate the average absorbance for each standard and sample well.

### Plot the Standard Curve

Plot absorbance *versus* concentration for standards S1-S8 using linear axes and fit the data with a quadratic equation.

### Determine the Sample Concentration

1. Use a calculator or spreadsheet to determine the quadratic regression line.
2. Use the equation of the curve generated by the regression fit to calculate the value of your samples.

### Spiked-Sample Recovery Calculation

$$\text{Recovery Factor} = \frac{10 \times \text{cpm of sample}}{\text{*Endothelin added to sample (cpm)}}$$

$$\text{Endothelin (pg) in purified sample} = \left[\frac{\text{Value from ELISA (pg/ml)}}{\text{Recovery Factor}}\right] \times \text{reconstituted volume of sample - added *Endothelin (pg)}$$

$$\text{Total Endothelin in sample (pg/ml)} = \frac{\text{Endothelin (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

## Performance Characteristics

### Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

| Endothelin (pg/ml) | Absorbance | |
|---|---|---|
| 250 | 1.382 | 1.384 |
| 125 | 0.749 | 0.774 |
| 62.5 | 0.372 | 0.379 |
| 31.3 | 0.180 | 0.167 |
| 15.6 | 0.088 | 0.085 |
| 7.8 | 0.054 | 0.052 |
| 3.9 | 0.040 | 0.046 |
| 0 | 0.028 | 0.026 |

**Table 1. Typical results**

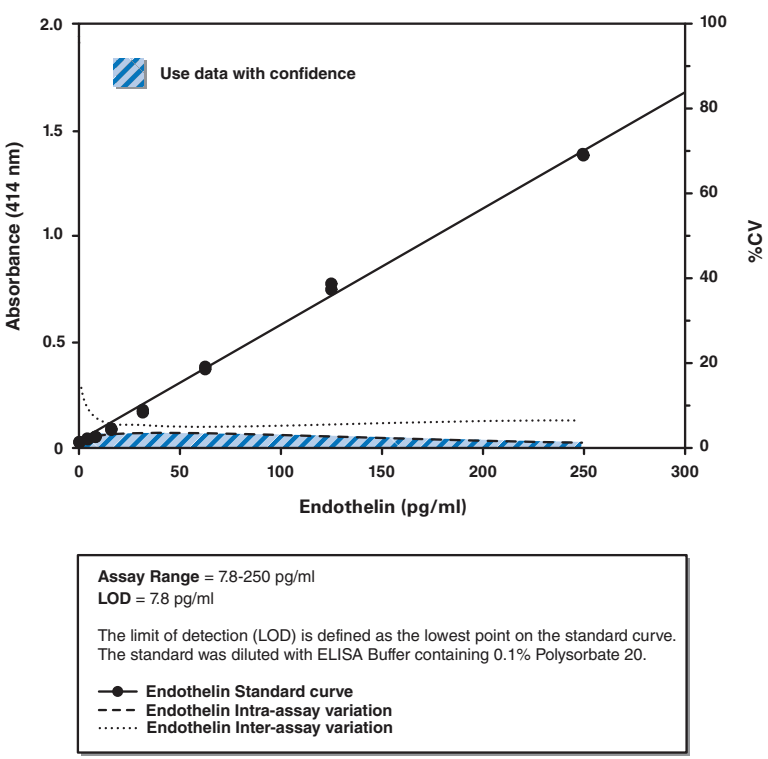


**Figure 7. Typical standard curve**

## Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the table below and in the graph on page 29.

| Dose (pg/ml) | %CV* Intra-assay variation | %CV* Inter-assay variation |
|---|---|---|
| 250 | 1.6 | 5.9 |
| 125 | 1.5 | 6.5 |
| 62.5 | 3.2 | 4.3 |
| 31.3 | 4.4 | 5.2 |
| 15.6 | 2.7 | 5.6 |
| 7.8 | 2.7 | 7.6 |
| 3.9 | 1.9 | 9.4 |
| 0 | 2.7 | 8.2 |

**Table 2. Intra- and inter-assay variation**

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

## Cross Reactivity:

| Compound | Cross Reactivity |
|---|---|
| Endothelin-1 | 100% |
| Endothelin-2 | 100% |
| Endothelin-3 | 100% |
| VIC | 100% |
| Big Endothelin | 100% |

**Table 3. Cross Reactivity of the Endothelin ELISA**

# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Trace organic contaminants in the water source<br>B. Poor pipetting/technique | A. Replace activated carbon filter or change source of UltraPure water |
| Poor development (low signal) of standard curve. | A. Standard was diluted incorrectly<br>B. Standard is degraded<br>C. Plate requires more development time | A. Replace activated carbon filter or change source of UltraPure water<br>B. Return plate to shaker and re-read later |
| Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference) | Interfering substances are present | A. Sample must be purified prior to analysis by ELISA[15]<br>B. Add mouse serum and DTT to standards and samples |
| Sample concentrations appear inconsistent with literature values | Matrix for samples and standards are different | A. Use same matrix for all samples and standards<br>B. Add mouse serum and DTT to standards and samples |



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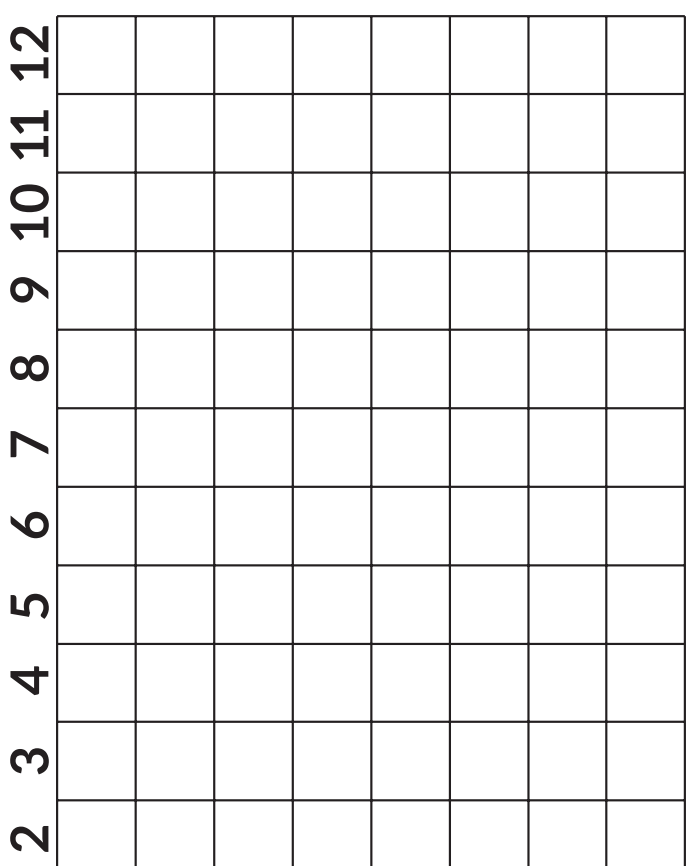




## NOTES

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