Resolvin E1 ELISA Kit

Item No. 502150

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	Storage Temperature
502151	Resolvin E1 ELISA Antiserum	1 vial/100 dtn	-20°C
502152	Resolvin E1 AChE Tracer	1 vial/100 dtn	-20°C
502153	Resolvin E1 ELISA Standard	1 vial/37.5 ng	-80°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
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 ea	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400040	ELISA Tracer Dye	1 ea	RT
400042	ELISA Antiserum Dye	1 ea	RT

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 Resolvin E1 ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

Researchers are advised to validate results from this ELISA with a secondary detection method or to clearly report the limitations of this ELISA in their publications.

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 as directed in the **Materials Supplied** section (see page 3) 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 at 405-420 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. 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).*
5. Materials used for **Sample Preparation** (see page 13).

INTRODUCTION

Background

Resolvin E1 (RvE1) is a member of the specialized pro-resolving mediator (SPM) family of bioactive lipids.[1] It is produced from eicosapentaenoic acid (EPA) by leukotriene A_4 (LTA_4) hydrolase in a two-step mechanism in human polymorphonuclear (PMN) neutrophils. EPA is converted to 18(R)-HEPE by aspirin-acetylated COX-2-mediated oxidation or microbial cytochrome P450-mediated oxidation. 18(R)-HEPE is then oxygenated by 5-lipoxygenase (5-LO) to generate RvE1 in human PMNs.[1,2] RvE1 has anti-inflammatory effects in various mouse models of inflammatory disease, including asthma, peritonitis, and colitis.[1,3,4] Levels of RvE1 are decreased in patients with inflammatory autoimmune diseases, including rheumatoid arthritis and Hashimoto's thyroiditis.[5,6]

About This Assay

Cayman's Resolvin E1 ELISA Kit is a competitive assay that can be used for the quantification of RvE1 in human plasma, serum, and urine. The assay has a range of 3.4-1,000 pg/ml, with a midpoint (50% B/B_0) of 40-60 pg/ml, and a sensitivity (80% B/B_0) of approximately 13 pg/ml.

Principle Of This Assay

This assay is based on the competition between free RvE1 and RvE1-acetylcholinesterase (AChE) conjugate (RvE1-AChE Tracer) for a limited number of RvE1 polyclonal antibody binding sites. Because the concentration of the RvE1-AChE Tracer is held constant while the concentration of free RvE1 varies, the amount of RvE1-AChE Tracer that is able to bind to the RvE1 Polyclonal Antibody will be inversely proportional to the concentration of free RvE1 in the well. This antibody-RvE1 complex binds to mouse anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 414 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of RvE1-AChE Tracer bound to the well, which is inversely proportional to the amount of free resolvin E1 present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{Bound RvE1-AChE}] \propto 1/[\text{RvE1}]$$

A schematic of this process is shown in Figure 1, on page 9.

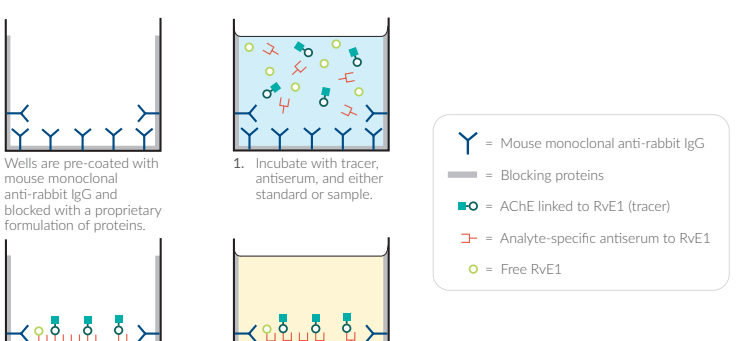


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the RvE1 AChE-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B0 values versus concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): 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/B0) value of the primary analyte when each is measured in assay buffer using the following formula:

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

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **ELISA Buffer (1X) 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.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

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

Testing for Interference

This assay has been validated in human plasma, serum, and urine. Other sample types should be tested 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 samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated RvE1 concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatibility in the assay.

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate for plasma samples. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Pipette off the top plasma layer without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Serum

Collect blood in vacutainers without a coagulant for serum samples. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1-2,000 x g for 15-30 minutes. Pipette off the serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Sample Purification

Human plasma and serum were purified using the following protocol. Alternative protocols may be used based on the experimental requirements, sample type, and the end user's expertise.

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended).
2. Add 4X the sample volume of ice-cold ethanol and vortex thoroughly. Incubate on ice for 5 minutes and centrifuge at 1,000 x g for 5 minutes. Transfer the supernatant to a clean test tube. Repeat this purification protocol one more time.*
3. Evaporate the ethanol under a gentle stream of nitrogen without heat.
4. Resuspend the extract in ELISA Buffer (1X) to its original volume, and use this for ELISA analysis.

*If it is necessary to stop during this purification, samples may be stored in the ethanol solvent stage at -20°C or -80°C.

Urine

Urine samples should be assayed immediately or stored at -20°C immediately after collection. Interference in urine is infrequent. Dilute urine samples with the ELISA Buffer (1X) to fall within the range of the standard curve. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701) or a similar assay.

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.
- Samples of rabbit origin may contain antibodies that interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples be purified prior to use in the assay.

Sample Matrix Properties

Parallelism

To assess parallelism, plasma, serum, and urine were processed as described in the **Sample Preparation** section (see page 13), serially diluted with ELISA Buffer (1X), and evaluated using the Resolvin E1 ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

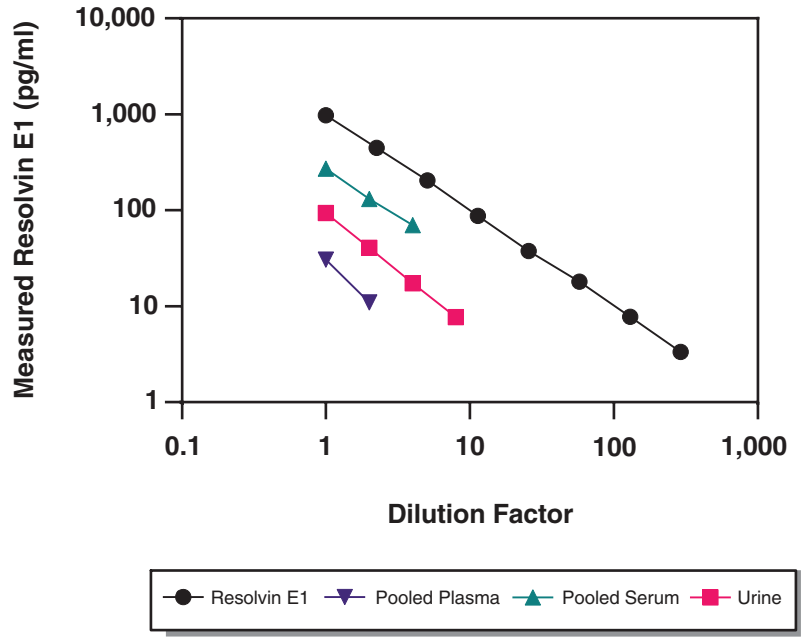


Figure 2. Parallelism of various matrices in the RvE1 ELISA

Spike and Recovery

Human plasma, serum, and urine were spiked with different amounts of RvE1, processed as described in the Sample Preparation section, serially diluted with ELISA Buffer (1X), and evaluated using the Resolvin E1 ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

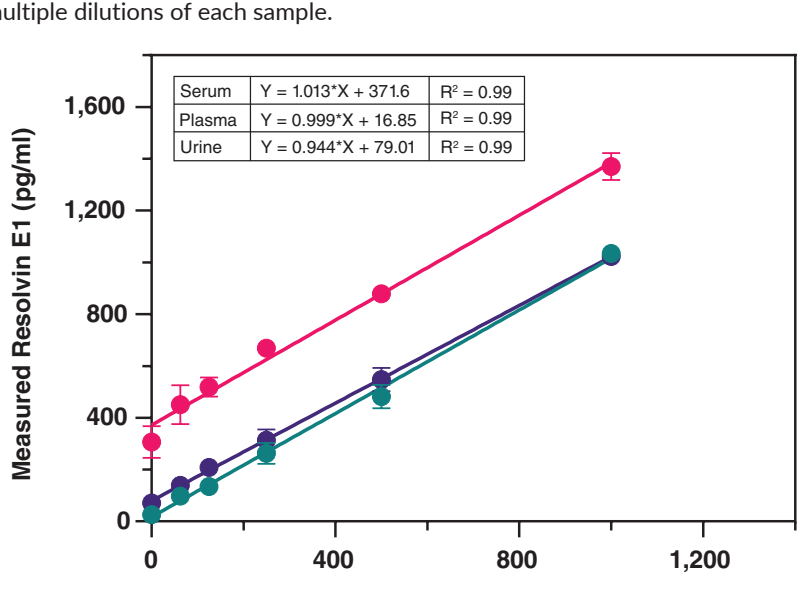


Figure 3. Spike and recovery of RvE1 in various matrices

Linearity

Human plasma, serum, and urine samples were spiked with RvE1 (125 pg/ml), processed as described in the Sample Preparation section, serially diluted with ELISA Buffer (1X), and evaluated for linearity using the Resolvin E1 ELISA Kit.

Dilution Factor	Measured Concentration (pg/ml)	Linearity (%)
Plasma		
2	152.9	100
4	133.1	87.1
Serum		
4	435.0	100
8	479.5	110.2
16	482.1	110.8
Urine		
4	393.3	100
8	409.0	104
16	480.8	122

Table 1. Linearity in various matrices

NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Preparation of Assay-Specific Reagents

Resolvin E1 ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Resolvin E1 ELISA Standard (Item No. 502153) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl of ultrapure water. The concentration of this solution (the bulk standard) will be 7.5 ng/ml.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 975 µl of ELISA Buffer (1X) to tube #1 and 500 µl of ELISA Buffer (1X) to tubes #2-8. Transfer 150 µl of the bulk standard (7.5 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

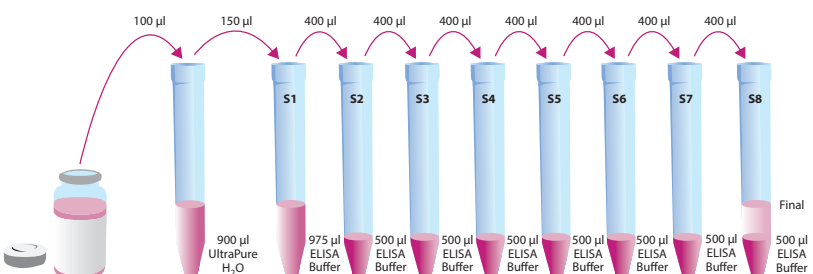


Figure 4. Preparation of the RvE1 standards

Resolvin E1-AChE Tracer

Reconstitute the Resolvin E1-AChE Tracer (Item No. 502152) with 6 ml of ELISA Buffer (1X). Store the reconstituted Resolvin E1-AChE Tracer at 4°C (*do not freeze!*). It will be stable for at least two weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer). *NOTE: Do not store tracer with dye for more than 2 weeks at 4°C.*

Resolvin E1 ELISA Polyclonal Antiserum

Reconstitute the Resolvin E1 ELISA Polyclonal Antiserum (Item No. 502151) with 6 ml of ELISA Buffer (1X). Store the reconstituted Resolvin E1 ELISA Polyclonal Antiserum at 4°C (*do not freeze!*). It will be stable for at least two weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

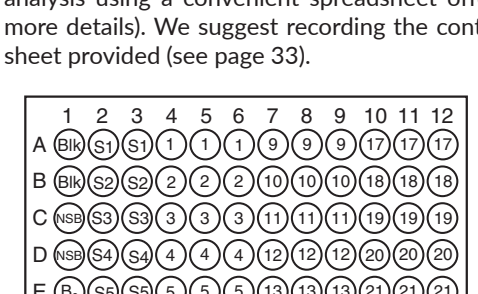
This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum). *NOTE: Do not store antiserum with dye for more than 2 weeks at 4°C.*

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 Blk, two NSB, and three B_0 wells, 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 a minimum of 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 page 24 for more details). We suggest recording the contents of each well on the template sheet provided (see page 33).



Blk = Blank Wells
TA = Total Activity Well
NSB = Non-Specific Binding Wells
B_0 = Maximum Binding Wells
S1-S8 = Standard Wells
1-24 = Sample Wells

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. **ELISA Buffer (1X)**

   Add 100 µl of ELISA Buffer (1X) to NSB wells. Add 50 µl of ELISA Buffer (1X) to B_0 wells.

2. **Resolvin E1 ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next 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.

3. **Samples**

   Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Resolvin E1 AChE Tracer**

   Add 50 µl to each well except the TA and Blk wells.

5. **Resolvin E1 ELISA Antiserum**

   Add 50 µl to each well, except the TA, NSB, and Blk wells, within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate overnight at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use. Reconstitute 100 dtn vial 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 be run on different days.

2. Empty the wells and rinse five times with ~300 µl of Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. Cover the plate with the 96-Well Cover Sheet. 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. This assay typically develops (*i.e.*, B_0 wells ≥0.6 A.U. (Blk subtracted)) in 90 minutes at room temperature.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Remove the cover sheet being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength of 414 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (Blk subtracted). The plate should be read when the absorbance of the B_0 wells is in the range of 0.3-1.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 1.5 A.U., 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 can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) 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.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* RvE1 concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be lineraized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any concentration of the sample prior to the addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

Performance Characteristics

Representative Data

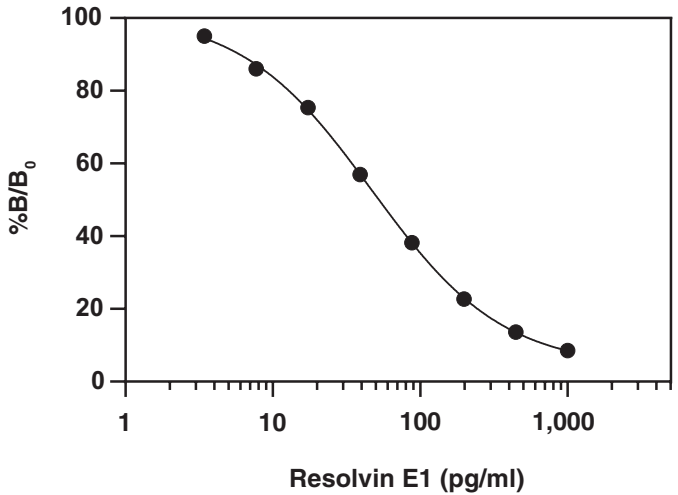
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

Absorbance at 414 nm (90 minutes)

RvE1 Standards (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.002	--	--	--	--
B_0	0.782	0.780	--	--	--
TA	2.642	--	--	--	--
1,000	0.068	0.066	8.5	13.7	7.8
444	0.108	0.106	13.3	5.9	4.2
198	0.179	0.177	22.7	5.7	4.8
87.8	0.300	0.298	38.2	5.4	2.6
39	0.446	0.444	57.0	10.5	7.4
17.3	0.590	0.588	75.4	12.9	5.9
7.7	0.673	0.671	86.0	29.3**	7.6
3.4	0.743	0.741	95.0	52.9**	10.0

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
**Evaluate data in this range with caution



Assay Range = 3.4-1,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 12.8 pg/ml
Mid-point (defined as 50% B/B_0) = 52.4 pg/ml
Lower Limit of Detection (LLOD) = 2.2 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in ELISA Buffer (1X).

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three spiked human plasma controls in a single assay.

Matrix Control (pg/ml)	%CV
719.4	9.6
466.8	7.8
147.8	12.3

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing three spiked human plasma controls in 9 nine separate assays on 4 different days.

Matrix Control (pg/ml)	%CV
660.9	13.7
375.6	12.3
142.4	9.4

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Resolvin E1	100%
Leukotriene B_4	0.025%
20-hydroxy Leukotriene B_4	0.008%
Resolvin E2	0.004%
(±)15-HEPE	<0.01%
(±)18-HEPE	<0.001%
Docosahexaenoic Acid	<0.001%
Eicosapentaenoic Acid	<0.001%
Protectin D1	<0.001%
Resolvin D1	<0.001%
Resolvin D2	<0.001%
Resolvin E4	<0.001%

Table 5. Cross Reactivity of the RvE1 ELISA

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>0.100)	A. Poor washing B. Exposure of NSB wells to specific antiserum
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose response curve)	A. Standard is degraded or contaminated B. Dilution error in preparing reagents
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Low signal in the sample wells (below the range of the standard curve)	A. AChE inhibitors are present; ensure that the samples and buffers are free of AChE inhibitors B. Sample requires further dilution
Only TA wells develop	A. Trace organic contaminants in the water source B. The tracer was not added to the wells

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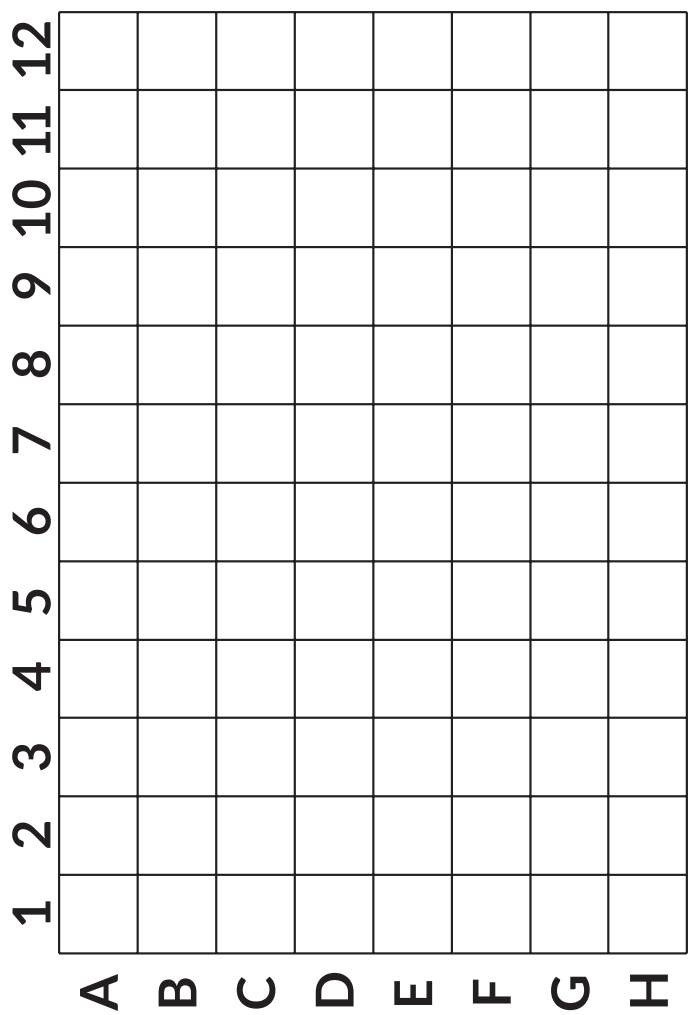
Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Reconstitute and Mix	Mix all reagents gently				
Add ELISA Buffer (1X)	--	--	100 µl	50 µl	--
Add Standards/Samples	--	--	--	--	50 µl
Add Resolvin E1-AChE Tracer	--	--	50 µl	50 µl	50 µl
Add Resolvin E1 ELISA Polyclonal Antiserum	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate overnight at 4°C				
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Add Ellman's Reagent	200 µl				
TA- Apply Tracer	--	5 µl	--	--	--
Develop	Seal the plate and incubate for 90 minutes at room temperature on an orbital shaker protected from light				
Read	Read absorbance at 414 nm				

Table 6. Assay Summary



NOTES

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