Estrone-3-Glucuronide ELISA Kit

Item No. 501290

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
501291	Estrone-3-Glucuronide ELISA Antiserum	1 vial/100 dtn
501292	Estrone-3-Glucuronide AP Tracer	1 vial/100 dtn
501293	Estrone-3-Glucuronide ELISA Standard	1 vial
400080	Tris Buffer Concentrate (10X)	1 vial/10 ml
411007	AP Wash Buffer Concentrate (150X)	1 vial/5 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate
400089	*p*NPP Substrate Solution	2 vials/12 ml
400040	ELISA Tracer Dye	1 ea
400042	ELISA Antiserum Dye	1 ea
400012	96-Well Cover Sheet	1 cover

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.

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.

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 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 at 405 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or deionized water is acceptable. *NOTE: Ultrapure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 11).

INTRODUCTION

Background

Estrone-3-glucuronide is the predominant metabolite of estradiol in urine and a urinary marker for the fertile window in women.[1] Measurement of urinary glucuronides is a convenient, non-invasive method for detecting reproductive hormone levels compared to measuring plasma levels.[2] Estrone-3-glucuronide, along with pregnanediol-3-glucuronide, are indicators of female reproductive health.[2]

Literature has shown high plasma estrone levels, which directly correlate to urinary estrone-3-glucuronide levels, are associated with estrogen receptor-positive breast cancers.[3] Hormone receptor status is the main factor in planning treatment and can be treated with hormone therapies, including tamoxifen and aromatase inhibitors. In addition to its role in breast cancer, circulating estrogens, including estrone metabolized from oral hormone replacement therapy, have been shown to increase thrombin generation leading to higher risk for blood clots, myocardial infarctions, deep vein thrombosis and strokes.[4-6]

About This Assay

Cayman's Estrone-3-Glucuronide ELISA Kit is a competitive assay that can be used for quantification of estrone metabolites in urine, plasma and serum. This assay has a range from 2.7-6,000 pg/ml with a midpoint of approximately 125.6 pg/ml (50% B/B_0) and a sensitivity of approximately 23.2 pg/ml (80% B/B_0).

Principle of the Assay

This assay is based on the competition between estrone-3-glucuronide and an estrone-3-glucuronide-alkaline phosphatase (AP) conjugate (Estrone-3-Glucuronide AP Tracer) for a limited amount of Estrone-3-Glucuronide ELISA Antiserum. Because the concentration of the Estrone-3-Glucuronide AP Tracer is held constant while the concentration of estrone-3-glucuronide standards and samples vary, the amount of Estrone-3-Glucuronide AP Tracer that is able to bind to the Estrone-3-Glucuronide ELISA Antiserum will be inversely proportional to the concentration of estrone-3-glucuronide in the well. This antibody-estrone-3-glucuronide complex binds to a mouse anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then pNPP Substrate Solution is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Estrone-3-Glucuronide AP Tracer bound to the well, which is inversely proportional to the amount of free estrone-3-glucuronide present in the well during the incubation; or

$$\text{Absorbance} \propto \text{[Bound Estrone-3-Glucuronide AP Tracer]} \propto 1/\text{[Estrone-3-Glucuronide]}$$

A schematic of this process is shown in Figure 1, on page 8

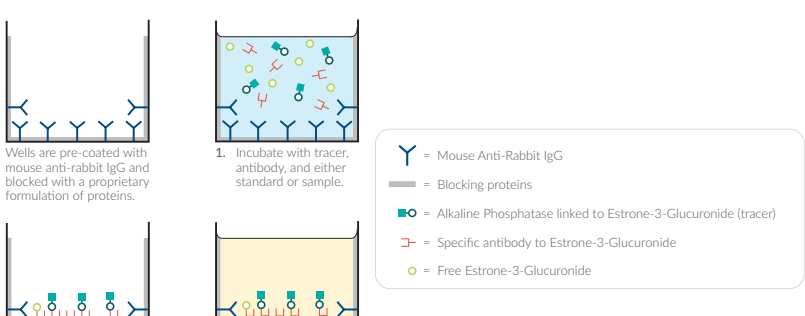


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blank: background absorbance caused by *p*NPP Substrate Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Total Activity (TA): total enzymatic activity of the alkaline phosphatase-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 particular sample or standard well to that of the maximum binding (B_0) well.

Standard Curve: a plot of the %B/B_0 values versus concentration of a series of wells containing various known amounts of analyte.

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:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% B/B}_0 \text{ value for the primary analyte}}{\text{50\% B/B}_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

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

PRE-ASSAY PREPARATION

Buffer Preparation

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

1. **Tris Buffer Preparation**

   Dilute the contents of one vial of Tris Buffer Concentrate (10X) (Item No. 400080) with 90 ml of Pure Water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **AP Wash Buffer Preparation**

   Dilute the contents of the vial of AP Wash Buffer Concentrate (150X) (Item No. 411007) to a total volume of 750 ml with Pure water. *NOTE: Smaller volumes of AP Wash Buffer can be prepared by diluting the concentrate 1:150.*

Sample Preparation

This assay has been demonstrated to work with urine, human plasma and human serum without extraction. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the 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 which interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples be purified prior to use in this assay.

Testing for Interference

This assay has been tested using human urine, plasma and serum. Other sample types will need to be assessed for interference by the end user. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between approximately 1,000 and 25 pg/ml (*i.e.*, between 20-80% B/B_0). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated estrone-3-glucuronide concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determine by the end user and tested for compatibility in the assay.

Sample Matrix Properties

Linearity

Human urine, plasma, and serum samples were spiked with estrone-3-glucuronide (100 ng/ml), processed as described in the Sample Preparation section, serially diluted with Tris Buffer (1X), and evaluated for linearity using the Estrone-3-Glucuronide ELISA Kit.

Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
Human Urine		
1:81	104.0	100.0
1:243	104.0	99.9
1:729	115.8	111.3
1:2,187	136.1	130.9
Human Plasma		
1:81	122.1	100.0
1:243	121.9	99.9
1:729	139.9	114.6
1:2,187	128.0	104.8
Human Serum		
1:81	97.0	100.0
1:243	93.3	96.2
1:729	112.3	115.8
1:2,187	116.7	120.4

Table 1. Dilutional Linearity of human urine, plasma, and serum samples.

Spike and Recovery

Human urine, plasma, and serum were spiked with estrone-3-glucuronide, diluted as described in the **Sample Preparation** section and analyzed using the Estrone-3-Glucuronide ELISA Kit. The results are shown below. The y-intercept corresponds to the amount of endogenous estrone-3-glucuronide in the sample. Error bars represent standard deviations obtained from multiple dilutions of each sample.

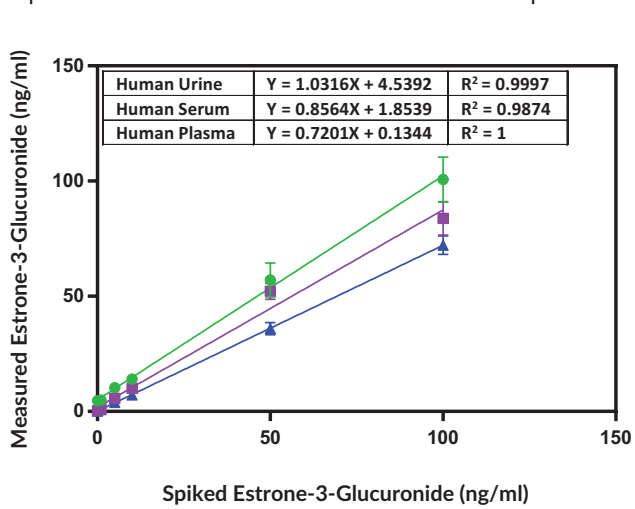


Figure 2. Spike and recovery in human urine, plasma, and serum

Sample	Spike Concentration (ng/ml)	% Recovery
Human Urine	5	112.5
	10	93.3
	50	104.6
	100	95.9
Human Plasma	1	90.8
	5	106.9
	10	98.0
	50	103.8
	100	83.5
Human Serum	1	62.0
	5	75.9
	10	71.5
	50	72.5
	100	72.1

Table 2. Spike and recovery of human urine, plasma, and serum samples.

Parallelism

To assess parallelism, human plasma, serum, and urine samples were checked at multiple dilutions using the Estrone-3-Glucuronide ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted human urine, plasma and serum samples.

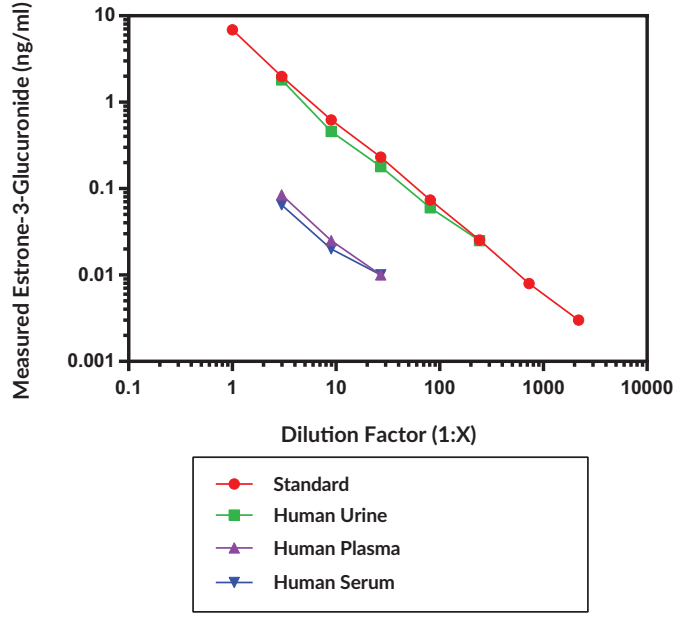


Figure 3. Parallelism of sample matrices in the Estrone-3-Glucuronide ELISA Kit

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Estrone-3-Glucuronide ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the supplied standard several times. Using the equilibrated pipette tip, transfer 100 µl of the Estrone-3-Glucuronide ELISA Standard (Item No. 501293) into a clean test tube, then dilute with 900 µl Pure Water. The concentration of this solution (the bulk standard) will be 60 ng/ml.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl 1X Tris Buffer to tube #1 and 400 µl Tris Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (60 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 200 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 200 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 two hours.

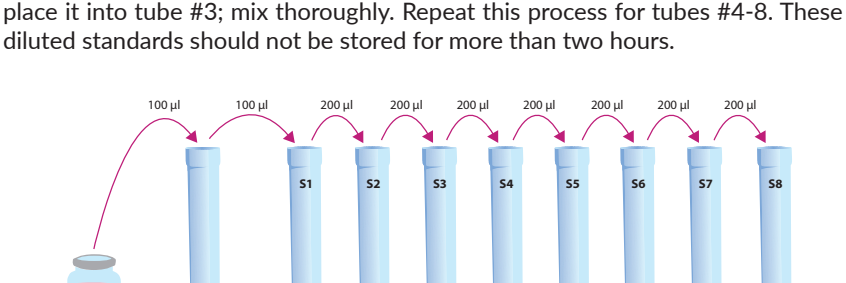


Figure 4. Preparation of the Estrone-3-Glucuronide standards

Estrone-3-Glucuronide AP Tracer

Reconstitute the Estrone-3-Glucuronide AP Tracer (Item No. 501292) with 6 ml of 1X Tris Buffer. Store the reconstituted Estrone-3-Glucuronide AP Tracer at 4°C (*do not freeze!*) and use within 4 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).

Estrone-3-Glucuronide ELISA Antiserum

Reconstitute the Estrone-3-Glucuronide ELISA Antiserum (Item No. 501291) with 6 ml of 1X Tris Buffer. Store the reconstituted Estrone-3-Glucuronide ELISA Antiserum at 4°C and use within 4 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).

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), two non-specific binding wells (NSB), two maximum binding wells (B_0), and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration 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 page 21, for more details). We suggest you record the contents of each well on the template sheet provided (see page 31).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - 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. **Tris Buffer (1X)**

   Add 100 µl Tris Buffer to Non-Specific Binding (NSB) wells. Add 50 µl Tris Buffer to Maximum Binding (B_0) wells.

2. **Estrone-3-Glucuronide 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 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.

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. **Estrone-3-Glucuronide AP Tracer**

   Add 50 µl to each well except the Total Activity (TA) and the Blank (Blk) wells.

5. **Estrone-3-Glucuronide ELISA Antiserum**

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

Incubation of the Plate

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

Rapid Protocol: Alternatively, you can incubate the plate for 2 hours on an orbital shaker at room temperature. (Please see Rapid Protocol section on page 26 prior to using this protocol

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl/well Wash Buffer.
2. Add 200 µl of *p*NPP Substrate Solution (Item No. 400089) to each well.
3. Dilute Tracer 1:10 in 1X Tris Buffer (i.e.; 10 µL reconstituted tracer into 90 µl Tris Buffer). Add 5 µl of the diluted tracer to the Total Activity (TA) wells.
4. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker for 90 minutes in the dark.

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 *p*NPP Substrate Solution from splashing on the cover. *NOTE: Any loss of pNPP Substrate Solution will affect the absorbance readings.*
3. Read the plate at a wavelength of 405 nm.

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 Chemical 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.)

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 versus estrone-3-glucuronide concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized 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 dilution 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

Sample Data

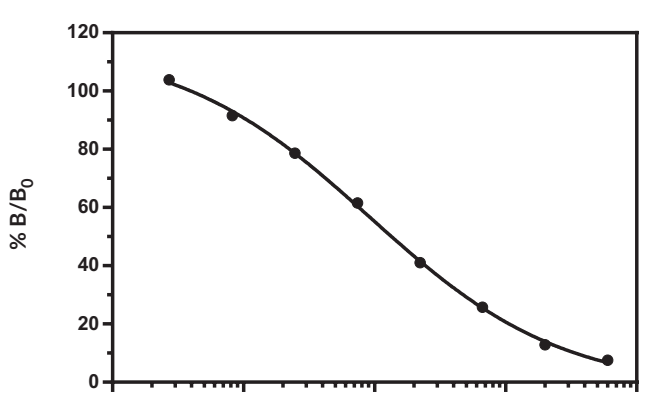
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the value of your samples.

Absorbance at 405 nm - 90 minute development - Overnight Incubation Step

Representative Data					
Estrone-3-Glucuronide Standards (pg/ml)	Blank Corrected Absorbance	NSB Corrected Absorbance	%B/B_0	%CV Intra-Assay Precision*	%CV Inter-Assay Precision
NSB	0.003	--	--	--	--
B_0	1.173	1.170	--	--	--
TA	0.501	0.498	--	--	--
6,000	0.090	0.087	7.5	5.0	9.2
2,000	0.172	0.169	12.8	4.3	5.1
666.7	0.305	0.302	25.8	5.0	6.4
222.2	0.484	0.481	41.1	5.5	6.1
74.1	0.724	0.721	61.5	4.6	3.8
24.7	0.926	0.923	78.7	8.9	5.2
8.2	1.076	1.073	91.5	21.9**	10.4
2.7	1.220	1.217	103.8	31.0**	12.6

Table 3. 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 = 2.7-6,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 23.2 pg/ml
Mid-point (defined as 50% B/B_0) = 125.6 pg/ml
Lower Limit of Detection (LLOD) = 6.6 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with EIA Buffer.

Figure 6. Typical standard curve (overnight)

Precision:

The intra-assay precision was determined by analyzing 24 replicates of three matrix controls (pooled human urine) in a single assay.

Intra-assay Precision	
Matrix Control (pg/ml)	%CV
1,015.7	8.2
71.7	11.2
11.6	17.6

Table 4. Intra-Assay Precision (overnight)

The inter-assay precision was determined by analyzing four replicate of three matrix controls (pooled human urine) in separate assays spanning across several days.

Inter-assay Precision	
Matrix Control (pg/ml)	%CV
1,209.0	10.1
93.6	14.1
17.5	13.9

Table 5. Inter-Assay Precision (overnight)

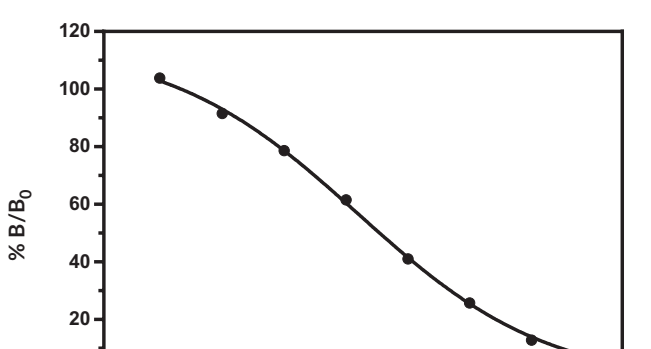
Rapid Protocol:

The Estrone-3-Glucuronide ELISA can be run in a rapid same-day assay format by simply incubating the plate for 2 hours instead of overnight as detailed in the "Incubation of the Plate" step on page 20 of this manual. The remaining steps of the protocol are identical in both the overnight and 2-hour assay format.

By utilizing the 2-hour assay format there will be a slight decrease in sensitivity in the assay, 80% B/B_0 of 36.1 pg/ml as compared to the overnight protocol of 80%B/B_0 of 23.2 pg/ml

Estrone-3-Glucuronide Standards (pg/ml)	Absorbance (blank and NSB corrected)	Average %B/B_0
B_0	0.986	
6,000	0.093	10.3
2,000	0.181	20.1
666.7	0.301	33.7
222.2	0.459	51.1
74.1	0.632	70.5
24.7	0.768	85.9
8.2	0.840	94.2
2.7	0.946	105.5

Table 6. Rapid Protocol



Assay Range = 2.7-6,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 36.1 pg/ml
Mid-point (defined as 50% B/B_0) = 246.8 pg/ml
Lower Limit of Detection (LLOD) = 10.1 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with EIA Buffer.

Figure 7. Typical standard curve (rapid protocol) 2-hour incubation step

Cross Reactivity:

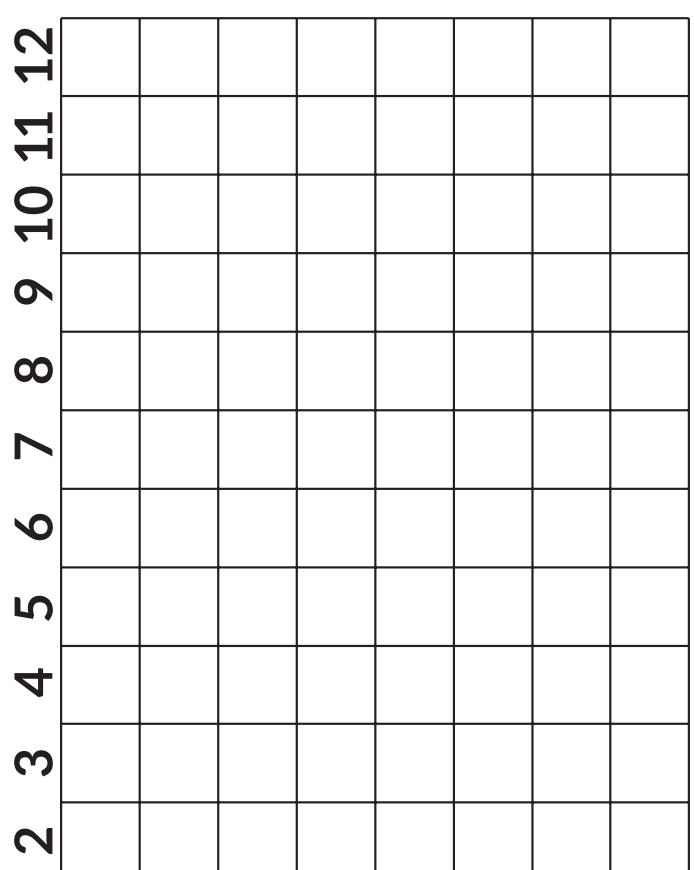
Compound	Cross Reactivity
Estrone-3-Glucuronide	100%
Estrone Sulfate	133%
Estrone	66%
Estradiol	0.13%
Estradiol Sulfate	0.1%
Progesterone	0.008%
Estriol	0.004%
DHEA Sulfate	0.0004%
Estriol Glucuronide	<0.01%
Testosterone Glucuronide	<0.01%
Estradiol Glucuronide	<0.01%
Pregnanediol Glucuronide	<0.01%

Table 7. Cross Reactivity of the Estrone-3-Glucuronide ELISA Kit

Assay Summary:

Procedure	Blk	TA	NSB	B_0	Standards/Samples
Reconstitute and Mix	Mix all reagents gently				
Tris Buffer (1X)	--	--	100 µl	50 µl	--
Estrone-3-Glucuronide Standards/Samples	--	--	--	--	50 µl
Estrone-3-Glucuronide AP Tracer	--	--	50 µl	50 µl	50 µl
Estrone-3-Glucuronide Antiserum	--	--	--	50 µl	50 µl
Seal	Seal the plate and tap gently to mix				
Incubate	Incubate plate overnight at 4°C or 2 hours at room temperature				
Aspirate	Aspirate wells and wash 5 x ~300 µl				
pNPP Substrate	200 µl	200 µl	200 µl	200 µl	200 µl
Total Activity (TA) - 1X Tracer diluted 1:10	--	5 µl	--	--	--
Seal	Seal plate and incubate for 90 min at room temperature on orbital shaker, protect from light				
Read	Read Absorbance at 405 nm				

Table 8. Estrone-3-Glucuronide Assay Summary



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 O.D.)	A. Poor washing, ensure proper washing is used B. Exposure of NSB wells to specific antibody
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)	Standard is degraded or contaminated
Analysis of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present - Consider sample purification prior to analysis.

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NOTES

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