ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Estriol
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K064-H1
5 Plate Kit Catalog Number K064-H5

Species Independent

Sample Types Validated:

Urine, Saliva, Dried Fecal Extracts, Extracted Serum/Plasma, and Tissue Culture Media

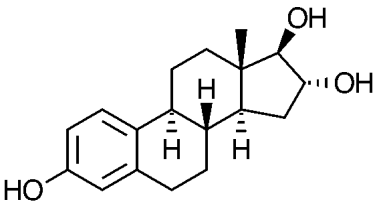
Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

BACKGROUND

Estriol is a major urinary estrogen. It is a hydroxylated metabolite of 17ß-estradiol or estrone with a hydroxyl group at the C3, 16α, and 17ß positions. As a metabolite of estrone, it is metabolized via 16α-hydroxyestrone through the enzyme 16α-hydroxysteroid dehydrogenase or to 2- or 4-hydroxyestrone by the action of catecho-O-methyltransferase. The latter metabolites can be formed in the brain and may compete with receptors for catecholamines. Prior to excretion by the kidney metabolites are conjugated with sulfate or glucuronide. During pregnancy, estriol constitutes 60-70% of the total estrogens, increasing to 300-500-fold in relation to non-pregnant women. The late term human fetus produces relatively large amounts of 16α-hydroxy DHEA, which serves the mother as a precursor of estriol. It has been shown 90% of the precursors for the formation of estriol are of fetal origin. If abnormal maternal serum screening results, specifically low levels of unconjugated estriol in the second trimester, a diagnosis of Smith-Lemli-Opitz syndrome (SLOS) may be suspected. SLOS is an autosomal recessive disorder caused by mutations of the gene encoding 7-dehydrocholesterol reductase.



Estriol

ASSAY PRINCIPLE

The DetectX® Estriol Immunoassay kit uses a specifically generated antibody to measure Estriol in urine and saliva, or in extracted serum, plasma and fecal samples. This kit is not recommended for serum or plasma samples without extraction. The kit will quantitatively measure estriol present in reconstituted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An estriol standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An estriol-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to estriol to each well.

We suggest 2 primary incubation times: 2-hours shaking at room temperature or at 4°C overnight prior to washing and addition of TMB substrate. The overnight protocol uses an identical standard curve range but is about 2-fold more sensitive.

After the primary incubation the plate is washed and substrate is added. The substrate reacts with the bound estriol-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the estriol in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Urinary Creatinine Detection Kits	K002-H1/H5
17-Hydroxyprogesterone EIA kits	K053-H1/H5
Aldosterone EIA & CLIA Kits	K052-H1/H5, -C1/C5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Dehydroepiandrosterone Sulfate (DHEA-S) EIA Kits	K054-H1/H5
Estradiol Non-Invasive & Serum EIA Kits	K030-H1/H5, KB30-H1/H5
Estrone-3-Glucuronide (E1G) EIA Kits	K036-H1/H5
Oxytocin EIA & CLIA Kits	K048-H1/H5, -C1/C5
PGFM (13,14-Dihydro-15-keto-Prostaglandin F_{1a}) EIA Kits	K022-H1/H5
Pregnandiol-3-Glucuronide (PDG) EIA Kits	K037-H1/H5
Progesterone EIA Kits	K025-H1/H5
Prolactin EIA Kit	K040-H1
Testosterone EIA Kits	K032-H1/H5

SUPPLIED COMPONENTS

Coated Clear 96 Well Plate
Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.

Kit K064-H1 **or** -H5 1 **or** 5 Each — Catalog Number X016-1EA

Estriol Standard
Estriol at 120,000 pg/mL in a special stabilizing solution.

Kit K064-H1 **or** -H5 125 µL **or** 625 µL — Catalog Number C236-125UL **or** -625UL

DetectX® Estriol Antibody
A color-coded rabbit polyclonal antibody specific for Estriol.

Kit K064-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C234-3ML **or** -13ML

DetectX® Estriol Conjugate
A color-coded Estriol-peroxidase conjugate in a special stabilizing solution.

Kit K064-H1 **or** -H5 3 mL **or** 13 mL — Catalog Number C235-3ML **or** -13ML

Assay Buffer Concentrate
A 5X concentrate that must be diluted with deionized or distilled water.

Kit K064-H1 **or** -H5 28 mL **or** 55 mL — Catalog Number X065-28ML **or** -55ML

Wash Buffer Concentrate
A 20X concentrate that must be diluted with deionized or distilled water.

Kit K064-H1 **or** -H5 30 mL **or** 125 mL — Catalog Number X007-30ML **or** -125ML

TMB Substrate
Kit K064-H1 **or** -H5 11 mL **or** 55 mL — Catalog Number X019-11ML **or** -55ML

Stop Solution
A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K064-H1 **or** -H5 5 mL **or** 25 mL — Catalog Number X020-5ML **or** -25ML

Plate Sealer
Kit K064-H1 **or** -H5 1 **or** 5 Each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C.
Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the Estriol Conjugate. This must be stored at -20°C.**

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Diethyl ether or ethyl acetate for extraction of serum or plasma samples.

Ethanol or methanol will be needed for extraction of fecal samples.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 µL.

A microplate shaker.

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

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

PRECAUTIONS

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

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

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

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

SAMPLE TYPES

This assay has been validated for urine and saliva samples, and extracted serum and plasma samples, dried fecal extracts and in tissue culture media . Samples containing visible particulate should be centrifuged prior to using. Estriol can be assayed in solid sample types or in serum and plasma samples by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols.

Estriol is identical across all species and we expect this kit to measure Estriol from all sources. The end user should evaluate recoveries of Estriol in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples

We have 3 detailed extraction options for liquid samples such as serum and plasma available on our website at www.arborassays.com/resources/#protocols. Pleaseselect the PDF entitled “Steroid Liquid Extraction Protocol”. We recommend using diethyl ether or ethyl acetate with the protocols.

Dried Fecal Samples

We have a detailed Extraction Protocol available on our website at: www.arborassays.com/resources/#protocols. The ethanol concentration in the final Assay Buffer dilution added to the well should be ≤ 5%.

Urine Samples

Urine samples should be diluted at least 1:8 in diluted Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection kits, K002-H1 and K002-H5.

Saliva Samples

Saliva samples should be diluted ≥ 1:4 in diluted Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions at www.arborassays.com/resources/#protocols.

Tissue Culture Media

For measuring Estriol in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

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

Assay Buffer

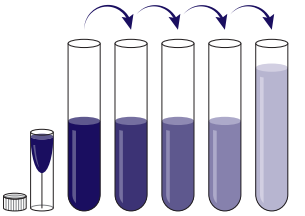
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

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

Standard Preparation

Label test tubes as #1 through #8. Pipet 270 µL of Assay Buffer into tube #1 and 150 µL into tubes #2 to #8. **The Estriol stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 30 µL of the Estriol stock solution to tube #1 and vortex completely. Take 100 µL of the Estriol solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of Estriol in tubes 1 through 8 will be 12,000, 4,800, 1,920, 768, 307.2, 122.9, 49.15 and 19.66 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**270**	150	150	150	150	150	150	150
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**30**	100	100	100	100	100	100	100
Final Conc (pg/mL)	12,000	4,800	1,920	768	307.2	122.9	49.15	19.66

Watch videos on sample preparation and setting up an assay on our website at:
www.arborassays.com/resources/#videos

ASSAY PROTOCOL

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

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 50 µL of samples or standards into wells in the plate.

3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.

4. Pipet 50 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.

5. Add 25 µL of the DetectX® Estriol Conjugate to each well using a repeater pipet.

6. Add 25 µL of the DetectX® Estriol Antibody to each well, **except the NSB wells**, using a repeater pipet.

7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer.

8. Incubation Options:

   EITHER:
   8a. Shake at room temperature for 2 hours. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 30% lower.

   OR:
   8b. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Incubate at 4°C for 16-18 hours without shaking.

9. If using Option 8b., the following day remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**

10. Aspirate the plate and wash each well 4 times with 300 µL Wash B uffer. Tap the plate dry on clean absorbent towels.

11. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

12. Incubate the plate at room temperature for 30 minutes without shaking.

13. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

15. Use the plate reader's built-in 4PLC software capabilities to calculate Estriol concentrations for each sample.

NOTE: Retain the plate frame for use with any remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-estriol-eia-kit.assay

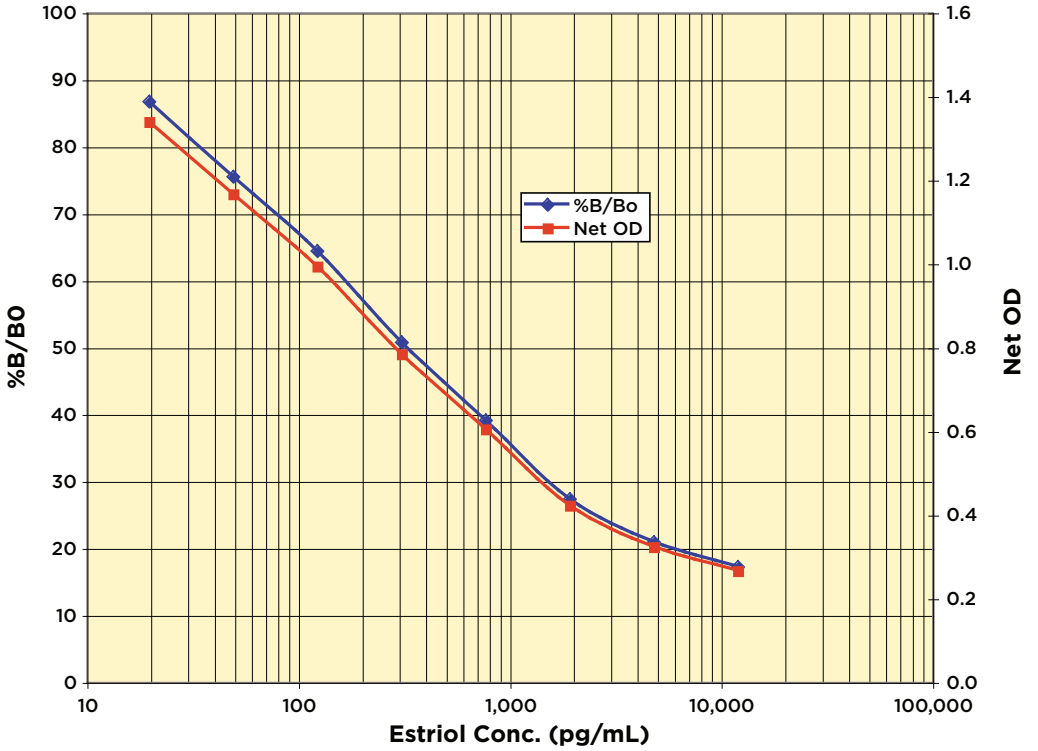
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Estriol Conc. (pg/mL)
NSB	0.084	0.000	-	-
Standard 1	0.351	0.267	17.3	12,000
Standard 2	0.408	0.324	21.0	4,800
Standard 3	0.507	0.423	27.4	1,920
Standard 4	0.689	0.605	39.2	786
Standard 5	0.869	0.785	50.8	307.2
Standard 6	1.079	0.995	64.4	122.9
Standard 7	1.251	1.167	75.6	49.15
Standard 8	1.424	1.340	86.8	19.66
B0	1.628	1.544	100	0
Sample 1	0.645	0.561	36.3	906.1
Sample 2	0.991	0.907	58.7	178.7

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

Conversion Factor: 100 pg/mL of Estriol is equivalent to 346.8 pM.

Typical Standard Curves



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

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 5.33 pg/mL.

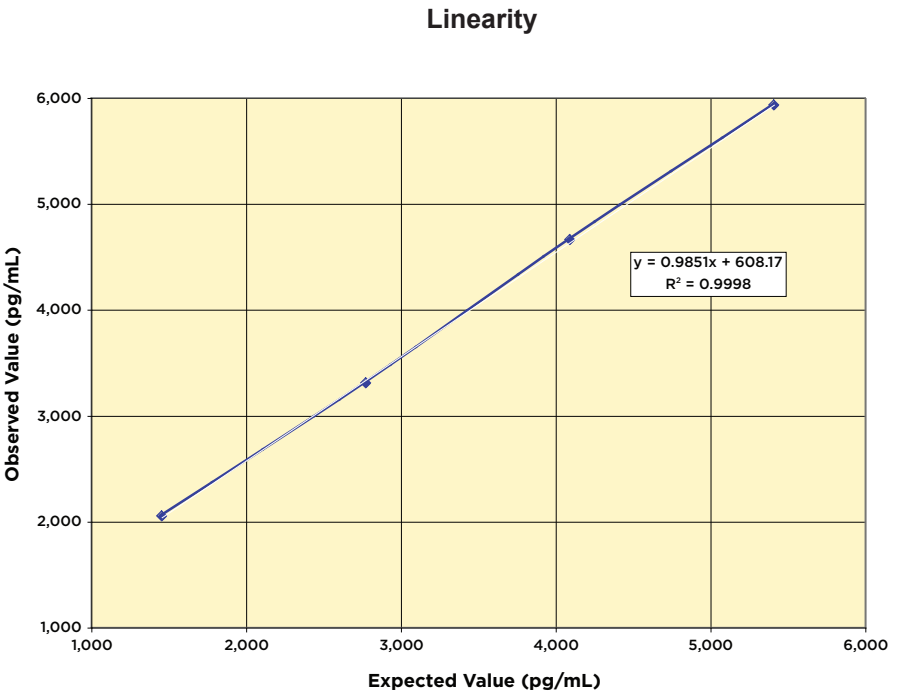
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human urine sample.

Limit of Detection was determined as 6.81 pg/mL.

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low estriol level of 135.5 pg/mL and one with a higher level of 6,729 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	5,410	5,933	109.7
60%	40%	4,091	4,661	113.9
40%	60%	2,773	3,308	119.3
20%	80%	1,454	2,054	141.2
			Mean Recovery	**121.0%**



Intra Assay Precision

Five human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Estriol concentrations were:

Sample	Estriol Conc. (pg/mL)	%CV
1	1,037	8.3
2	186.2	11.3
3	72.72	13.1
4	634.8	8.4
5	126.6	8.4

Inter Assay Precision

Five human samples were diluted with Assay Buffer and run in duplicates in twenty assays run over multiple days by multiple operators. The mean and precision of the calculated Estriol concentrations were:

Sample	Estriol Conc. (pg/mL)	%CV
1	1,015	10.1
2	196.1	11.6
3	81.84	17.5
4	631.8	10.5
5	123.2	11.0

SAMPLE VALUES

Human urine samples were tested in the assay. Adjusted neat concentrations of Estriol for the urine samples from non-pregnant females and males ranged from 1.7 to 66.2 ng/mL. Three urine samples from pregnant females read between 1,097 to 2,076 ng/mL.

Extracted fecal samples from a variety of mammals read from undetectable to as high as 7,233 pg/mL.

Two saliva samples from pregnant females read at 172 and 2,225 pg/mL. Samples from non-pregnant females had undetctable levels of estriol.

For urine samples we recommend the use of a kit to measure creatinine to normalize for urine output such as the DetectX® Urinary Creatinine detection kit, K002-H1 and K002-H5.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)	Steroid	Cross Reactivity (%)
Estriol	100%	Cortisone	< 0.01%
Estriol 3-glucuronide	57.16%	Desoxycorticosterone	< 0.01%
Estriol 3-sulphate	38.50%	DHEA	< 0.01%
16-Epiestriol	6.77%	DHEA-S	< 0.01%
17ß-Estradiol	0.03%	DHT	< 0.01%
17-Epiestriol	0.02%	17a-Estradiol	< 0.01%
Androstenedione	< 0.01%	Estrone	< 0.01%
Androsterone	< 0.01%	Ethynylestradiol	< 0.01%
Corticosterone	< 0.01%	Progesterone	< 0.01%
Cortisol	< 0.01%	Testosterone	< 0.01%

LIMITED WARRANTY

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

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

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

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

ASSAYS™
FOR SERIOUS SCIENCE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12