



ARBOR ASSAYS™
Interactive Assay Solutions™

ISWE

**17 β -Estradiol
Mini-Kit**

Catalog Number ISWE008

Species Independent

Official Supplier
to ISWE

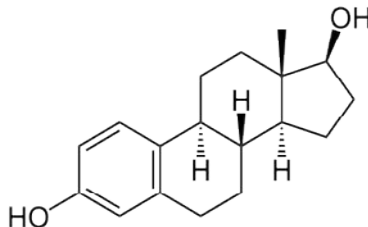


Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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MINI-KIT PRINCIPLE

The ISWE 17 β -Estradiol Mini-Kit is specifically designed to measure estradiol and/or its metabolites present in a variety of samples and species for wildlife conservation. The end user must provide or coat their own anti-sheep IgG secondary antibody coated plates, in addition to providing assay buffer, wash buffer, substrate and stop solution. On page 3, there is a list of Related Materials we supply that are used in our kits.



Three materials are supplied in the ISWE-Kit:

1. The ISWE 17 β -Estradiol sheep antibody was raised to the 6-CMO derivative of estradiol. The antibody has been titered and diluted in a buffer containing protein, detergents and Kathon preservative (see below) to ensure its stability. It is at a nominal "50X" concentration*.

NOTE: The 50X Concentrate Sheep Antibody to Estradiol cannot be directly coated onto plates for EIA use. It is unpurified serum diluted in a buffer containing proteins and detergents. The antibody WILL NOT bind to uncoated microtiter plates effectively as the antiserum proteins and the dilution buffer both contain high concentrations of non-specific proteins that will coat any plate surface.

2. The ISWE 17 β -Estradiol-Peroxidase conjugate is synthesized from 6-CMO derivative of estradiol and has been diluted in a stabilizing solution (see below) to ensure long term stability. It is at a nominal "50X" concentration*.
3. The 17 β -Estradiol Standard is synthetic steroid in a stabilizing solution containing buffer and organic solvent. It is supplied at 1,000 ng/mL for dilution into the assay buffer used for your assay.

* The nominal "50X Concentration" the ISWE Antibody and Conjugate are supplied at was determined using Arbor Assays 17 β -Estradiol EIA kit reagents from K030-H1 and K030-H5. The ISWE Antibody was diluted in Assay Buffer, from X065, at 1:50 and the ISWE Conjugate diluted in Conjugate Diluent, X076, at 1:50. The assay utilized 100 μ L sample, 50 μ L antibody and 50 μ L conjugate added to donkey anti-sheep IgG coated plate, X061-1EA. Assay incubation time was 2 hours shaking at room temperature, followed by washing and addition of 100 μ L of TMB Substrate. After 30 minutes the substrate reaction was stopped by addition of 50 μ L of Stop Solution. The color was read at 450 nm.

SUPPLIED COMPONENTS

ISWE 17 β -Estradiol Antibody Concentrate

A 50X Concentrate sheep polyclonal antibody specific for estradiol diluted in a buffer with proteins, detergents and preservatives.

6 mL

Catalog Number C266-6ML

ISWE 17 β -Estradiol Conjugate Concentrate

A 50X Concentrate estradiol-peroxidase conjugate in a special stabilizing solution.

6 mL

Catalog Number C267-6ML

17 β -Estradiol Standard

17 β -Estradiol at 1,000 ng/mL in a special stabilizing solution.

1 mL

Catalog Number C268-1ML

STORAGE INSTRUCTIONS

All components of this kit should be aliquoted into vials upon first thaw. Avoid repeated freeze thaw cycles. Store at -20°C until the expiration date of the kit.

RELATED MATERIALS

Description	Catalog No.
Donkey anti-Sheep IgG Coated Plate	X061-1EA
Assay Buffer 5x Concentrate	X065-110ML
HRP Conjugate Diluent	X076-300ML
Wash Buffer 20x Concentrate	X007-125ML
TMB Substrate	X019-55ML
Stop Solution	X020-25ML
Kathon Preservative	X129-25ML
Plate Coating Buffer 20x Concentrate	X108-100ML
Blocking Buffer 10x Concentrate	X109-250ML
Donkey anti-Sheep IgG, Affinity Purified	A010-10MG/-25MG/-50MG/-100MG
Arbor Assays ISWE Plate Coating Course	Contact us for details

PRECAUTIONS

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**.

PROTOCOLS

Please Note: The dilution of the ISWE Mini-Kit antibody and conjugate are such that when diluted 1:50 fold in Assay Buffer and Conjugate Diluent and added to an Arbor Assays antibody coated plate, using 100 μL of sample or standards, 50 μL of conjugate and 50 μL of antibody in a 2 hour room temperature incubation with shaking (we recommend shaking around 700–900 rpm) followed by TMB as substrate, these reagents will generate over 1.0 OD at 450 nm. **YOUR RESULTS MAY VARY DEPENDING ON A NUMBER OF VARIABLES.**

The sheep anti-estradiol is supplied at a 50X concentration. An aliquot should be brought to room temperature and diluted one part with 49 parts of a suitable assay buffer. If your assay buffer has protein, detergent and a preservative in it, the diluted antibody will be stable for greater than 1 month at 4°C. Please see Related Materials on page 3 for a suitable Assay Buffer.

The estradiol-HRP conjugate is supplied at a 50X concentration. An aliquot should be brought to room temperature and diluted one part with 49 parts of a suitable diluent or assay buffer. If you wish to store the diluted conjugate for greater than 1 month at 4°C you should dilute it in a suitable conjugate diluent. Please see Related Materials on page 3 for a suitable Conjugate Diluent.

The 17 β -estradiol standard stock is provided in a solution that contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.

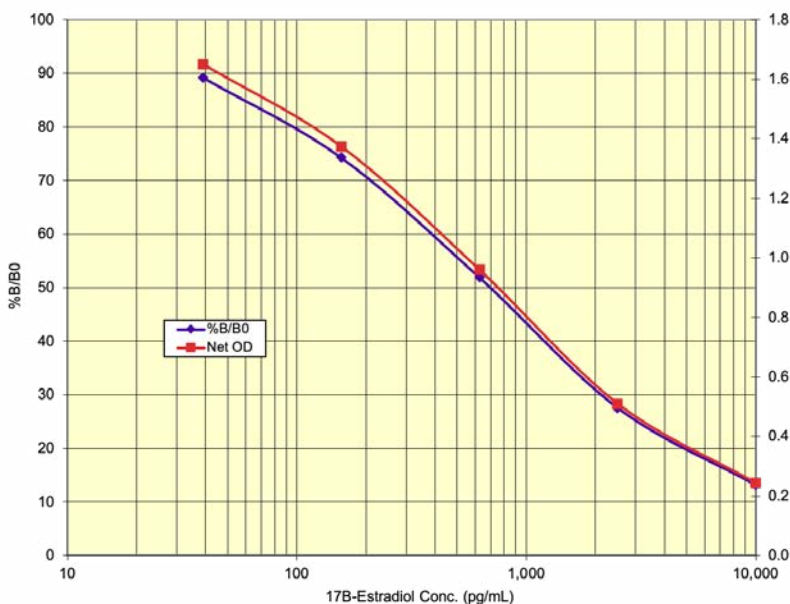


ARBOR
ASSAYS

TYPICAL DATA USING ARBOR ASSAYS COMPONENTS

Sample	Mean OD	Net OD	% B/B0	17 β -Estradiol Conc. (pg/mL)
NSB	0.122	0.000	---	-
Standard 1	0.365	0.243	13.14	10,000
Standard 2	0.632	0.510	27.57	2,500
Standard 3	1.082	0.960	51.89	625
Standard 4	1.496	1.374	74.27	156.25
Standard 5	1.773	1.651	89.24	39.06
B0	1.972	1.850	100.00	0
Sample 1	0.819	0.697	37.68	1,346.9
Sample 2	0.988	0.866	46.78	805.1

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



CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
17 β -Esteradiol	100
Estrone	3.52
Estrone-3-Sulfate	8.15
17a-Estadiol	0.12
Testosterone	0.04
Cortisol	< 0.01
Corticosterone	< 0.01
Progesterone	< 0.01

SAMPLES

The kit has been tested with a number of fecal samples from a diverse range of species, such as:



Tamandua



Sumatran Tiger



Hartmann's Zebra



Southern White
Rhinoceros

NOTE: Only preliminary testing has been carried out and the results are unpublished. The responsibility for validating the kit for various species, sample types and applications is the end user's. ISWE recommends that for every species and sample type, both laboratory (recovery and parallelism tests) and biological validations be conducted. References for validation methods can be obtained on the ISWE website, www.iswe-endo.org/resources/, or at admin@iswe-endo.org. Contact Arbor Assays at technical@arborassays.com with your results using this kit so that we may pass on information about the use of the kit. Reported use of this kit can also be found on WEIN, www.iswe-endo.org/wein/.

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:

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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.

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