

ARBOR ASSAYS™
Interactive Assay Solutions™

ISWE

**Cortisol
Mini-Kit**

Catalog Number ISWE002

Species Independent

Official Supplier
to ISWE



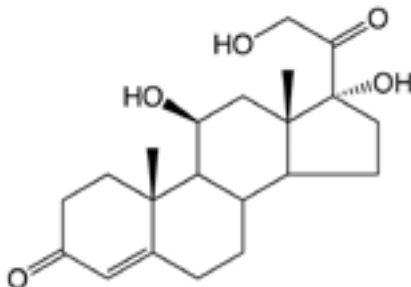
Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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ISWE002 WEB 210226

MINI-KIT PRINCIPLE

The ISWE Cortisol Mini-Kit is specifically designed to measure cortisol 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-rabbit IgG secondary antibody coated plates, in addition to providing assay buffer, wash buffer, substrate and stop solution. There is a list of Related Materials we supply that are used in our kits on page 3.



Three materials are supplied in the ISWE-Kit:

1. The ISWE Cortisol rabbit antibody, developed and owned by ISWE, was raised to the 3-CMO derivative of cortisol. 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 Rabbit Antibody to Cortisol 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 Cortisol-Peroxidase conjugate is synthesized from 3-CMO derivative of cortisol and has been diluted in a stabilizing solution (see below) to ensure long term stability. It is at a nominal "50X" concentration*.
3. The Cortisol Standard is synthetic steroid in a stabilizing solution containing buffer and organic solvent. It is supplied at 320 ng/mL for dilution into the assay buffer used for your assay.

* The nominal "50X Concentration" the ISWE Antibody and Conjugate are QC tested using Arbor Assays Cortisol EIA kit reagents from K003-H1 and K003-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 50 μ L sample, 25 μ L antibody and 25 μ L conjugate added to a goat anti-rabbit IgG coated plate, X016-1EA. 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 Cortisol Antibody Concentrate

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

6 mL

Catalog Number C208-6ML

ISWE Cortisol Conjugate Concentrate

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

6 mL

Catalog Number C209-6ML

Cortisol Standard

Cortisol at 320 ng/mL in a special stabilizing solution.

1 mL

Catalog Number C210-1ML

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.
Goat anti-Rabbit IgG ISWE Plate Coating Accessory Kit	ISWE005
Assay Development ISWE Accessory Kit	ISWE006
Goat anti-Rabbit IgG Coated Plate	X016-1EA
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
Dissociation Reagent	X017-5ML/-25ML
Kathon Preservative	X129-25ML
Plate Coating Buffer 20x Concentrate	X108-100ML
Blocking Buffer 10x Concentrate	X109-250ML
Goat Anti-Rabbit IgG, Affinity Purified	A009-10MG
Goat Anti-Rabbit IgG, Affinity Purified	A009-25MG
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 50 μ L of sample or standards, 25 μ L of conjugate and 25 μ L 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 about 1.0 OD at 450 nm. **YOUR RESULTS MAY VARY DEPENDING ON A NUMBER OF VARIABLES.**

The rabbit anti-cortisol 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 cortisol-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.

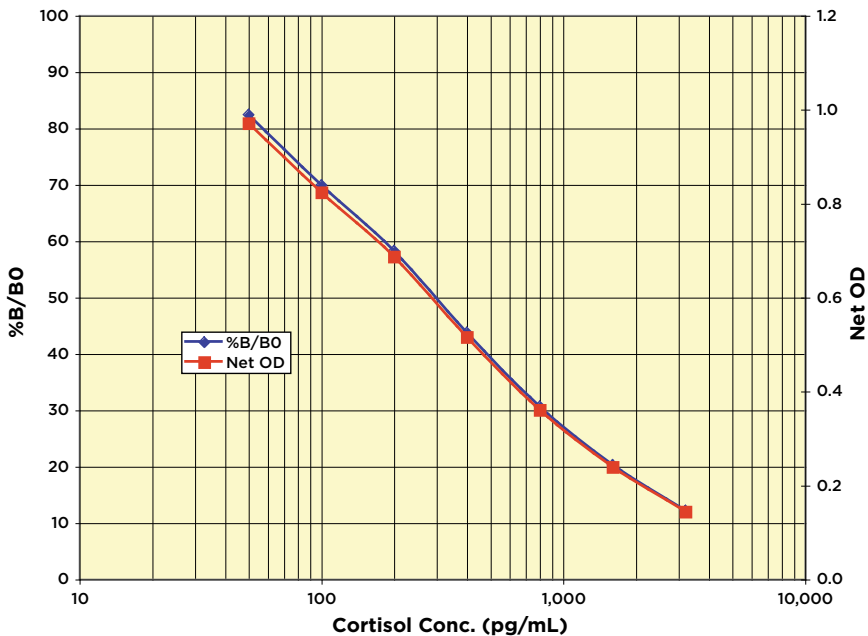
The cortisol standard stock is provided in a solution that contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.



TYPICAL DATA USING ARBOR ASSAYS COMPONENTS

Sample	Mean OD	Net OD	% B/B0	Cortisol Conc. (pg/mL)
NSB	0.085	0.000	---	-
Standard 1	0.229	0.144	12.22	3,200
Standard 2	0.324	0.239	20.29	1,600
Standard 3	0.446	0.361	30.65	800
Standard 4	0.601	0.516	43.80	400
Standard 5	0.772	0.687	58.32	200
Standard 6	0.909	0.824	69.95	100
Standard 7	1.056	0.971	82.43	50
B0	1.263	1.178	100	0
Sample 1	0.411	0.326	27.67	973.2
Sample 2	0.674	0.589	50.0	292.2

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 (%)
Cortisol	100%
Dehydrocortisol	42.08%
Cortisone	26.53%
Dexamethasone	4.10%
Prednisone	3.37%
Corticosterone	0.35%
Desoxycorticosterone	0.18%
Tetrahydrocorticosterone	< 0.16%

SAMPLES

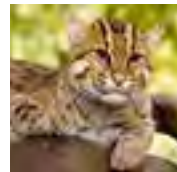
The kit has been tested with a number of fecal samples from a diverse range of species. These are:



Tiger



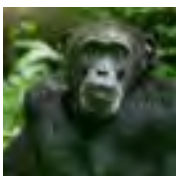
Red Panda



Fishing Cat



Clouded Leopard



Chimpanzee



Dugong

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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