

ARBOR ASSAYS™
Interactive Assay Solutions™

ISWE

**11-Oxoetiocholanolone (72T)
Mini-Kit**

Catalog Number ISWE010

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.

www.ArborAssays.com   

WEB ISWE010 231009

MINI-KIT PRINCIPLE

The ISWE 11-Oxoetiocholanolone (72T) Mini-Kit is specifically designed to measure a group of glucocorticoid metabolites with a 5β - 3α - 11 -one configuration 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. 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 Mini-Kit:

1. The ISWE 11-Oxoetiocholanolone rabbit antibody was raised from the respective 17-CMO:BSA derivative of 72T. 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 72T cannot be directly coated onto plates for EIA use. It is unpurified rabbit 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 11-Oxoetiocholanolone Peroxidase conjugate was synthesized from the respective 17-CMO derivative of 72T and has been diluted in a stabilizing solution (see below) to ensure long term stability. It is at a nominal "50X" concentration*.
3. The 11-Oxoetiocholanolone Standard is synthetic steroid in a stabilizing solution containing buffer and organic solvent. It is supplied at 4,000 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 EIA reagents. The ISWE Antibody was diluted in 1X Assay Buffer, from X065, at 1:50 and the ISWE Conjugate diluted in Conjugate Diluent, X076, at 1:50. The assay utilized 100 uL sample, 50 uL antibody and 50 uL conjugate added to 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 uL of TMB Substrate. After 30 minutes the substrate reaction was stopped by addition of 50 uL of Stop Solution. The color was read at 450 nm.



SUPPLIED COMPONENTS

ISWE 72T Antibody 50x Concentrate

A 50X Concentrate rabbit polyclonal antibody specific for 11-oxoetiocholanolone diluted in a buffer with proteins, detergents and preservatives.

6 mL

Catalog Number C327-6ML

ISWE 72T-HRP Conjugate 50x Concentrate

A 50X Concentrate 11-oxoetiocholanolone-peroxidase conjugate in a special stabilizing solution.

6 mL

Catalog Number C328-6ML

72T Standard

11-Oxoetiocholanolone at 4,000 ng/mL in a special stabilizing solution.

1 mL

Catalog Number C312-1ML

STORAGE INSTRUCTIONS

All components of this kit must be aliquoted into single use volumes 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 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 (1N HCl) | X020-25ML |
| 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/-23MG/-50MG/-100MG |
| Arbor Assays ISWE Plate Coating Course | Contact us for details |
| Assay Development ISWE Accessory Kit | ISWE006 |
| Goat anti-Rabbit IgG ISWE Plate Coating Accessory Kit (10 Plate) | ISWE005 |

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

The rabbit anti-72T 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 at least 1 week at 4°C. Please see Related Materials on page 3 for a suitable Assay Buffer.

The 72T-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 at least 1 week 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 72T standard stock is provided in a solution that contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.

Incubate 100µL of sample or standards, 50µL of diluted conjugate, and 50µL of diluted antibody for two hours at room temperature with shaking (recommend 700-900 rpm). Wash the plate. Add TMB as substrate and quench with Stop Solution. The 50-fold diluted ISWE Mini-kit antibody and conjugate with an Arbor Assays antibody coated plate will generate above 1.0 OD at 450 nm. **YOUR RESULTS MAY VARY DEPENDING ON A NUMBER OF VARIABLES.**

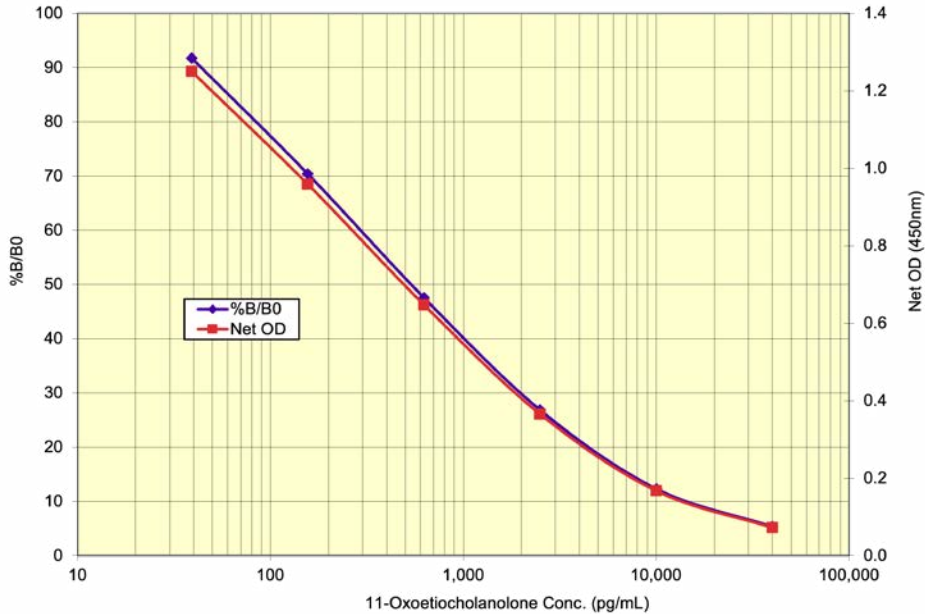


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TYPICAL DATA USING ARBOR ASSAYS COMPONENTS

| Sample | Mean OD | Net OD | % B/B0 | 72T Conc. (pg/mL) |
|------------|---------|--------|--------|-------------------|
| NSB | 0.081 | 0.000 | - | - |
| Standard 1 | 0.153 | 0.073 | 5.3 | 40,000 |
| Standard 2 | 0.248 | 0.168 | 12.3 | 10,000 |
| Standard 3 | 0.446 | 0.366 | 26.8 | 2,500 |
| Standard 4 | 0.728 | 0.648 | 47.6 | 625.00 |
| Standard 5 | 1.039 | 0.959 | 70.4 | 156.25 |
| Standard 6 | 1.330 | 1.250 | 91.8 | 39.06 |
| B0 | 1.442 | 1.362 | 100.00 | 0 |

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:

| Molecule | Cross Reactivity (%) |
|---|----------------------|
| 5 β -3 α -hydroxyandrostan-11,17-dione (11-Oxoetiocholanolone) | 100.00% |
| 5 β -Pregnan-3 α -ol-11,20-dione (Alloalfaxolone) | 9.62% |
| 5 β -Androstan-3,1,17-trione | 4.75% |
| 5 β -Androstan-3 α ,11B-diol-17-one (11B-Hydroxyeticholanolone) | 0.99% |
| 5 β -Pregnan-3 α ,11B,21-triol-20-one (Tetrahydrocorticosterone) | 0.20% |
| 5 β -Pregnan-3 α ,11 β -diol-20-one | 0.09% |
| Cortisone | 0.06% |
| Corticosterone | <0.04% |
| Cortisol | <0.04% |
| 17 β -Estradiol | <0.04% |
| Progesterone | <0.04% |
| Testosterone | <0.04% |

SAMPLES

The kit has been tested in feces from a diverse range of species. Including:

African elephant,
Loxodonta africana

Cape Buffalo,
Syncerus Caffer

Mandrill,
Mandrillus Sphinx

Ghost Bat,
Macroderma Gigas

Asian elephant,
Elephas maximus

Giraffe,
Giraffa Camelopardalis

Golden Langur,
Trachypithecus Geei

Brushtail Possum,
Trichosurus Vulpecula

Okapi,
Okapia johnstoni

Roan Antelope, Hippotragus
Equinus

Samango Monkey,
Cercopithecus Albogularis

Echidna,
Tachyglossus Aculeatus

Florida manatee,
Trichechus manatus latirostri

Chamois, Rupicapra Rupicapra

Fat-Tailed Dunnart,
Sminthopsis Crassicaudata

Blue-And-Yellow Macaw, Ara
Ararauna

Hippopotomus,
Hippopotamus amphibius

Tiger, Panthera Tigris

Gould's Wattled Bat,
Chalinolobus Gouldii

Blue wildebeest,
Connochaetes taurinus

Gorilla,
Gorilla Gorilla Gorilla

Leadbeater's Possum,
Gymnobelideus Leadbeateri

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/.



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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.

The Arbor Assays logo is a registered trademark.

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