

ARBOR ASSAYS™  
Interactive Assay Solutions™



# ISWE

## Assay Development Kit

Catalog Number ISWE006

Official Supplier  
to ISWE



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

# ASSAY DEVELOPMENT ACCESSORY KIT

This kit is designed as an accessory kit for wildlife researchers who wish to have stable, reproducible assays for measuring steroids.

## SUPPLIED COMPONENTS

### Conjugate Diluent

A diluent designed to stabilize peroxidase conjugated steroids for immunoassays.  
4 Each of 13 mL                      Catalog Number X076-13ML

### Assay Buffer Concentrate

A 5X Concentrate of Assay Buffer containing BSA and detergent with preservative.  
2 Each of 55 mL                      Catalog Number X065-55ML

### Wash Buffer Concentrate

A 20X Concentrate of Wash Buffer with preservative.  
2 Each of 125 mL                      Catalog Number X007-125ML

### TMB Substrate

2 Each of 55 mL                      Catalog Number X019-55ML

### Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**  
2 Each of 25 mL                      Catalog Number X020-25ML

## STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

## RELATED MATERIALS

Description	Catalog No.
Goat anti-Mouse IgG Plate Coating Kit, 10 Plates	ISWE004
Goat anti-Rabbit IgG Plate Coating Kit, 10 Plates	ISWE005
Goat anti-Mouse IgG Coated Plate	X012-1EA
Goat anti-Rabbit IgG Coated Plate	X016-1EA
Dissociation Reagent	X017-5ML/-25ML
Kathon Preservative	X129-25ML
Coating Buffer 10x Concentrate	X108-100ML
Blocking Buffer 10x Concentrate	X109-250ML
Goat anti-Mouse IgG, Affinity Purified	A008-10MG/-25MG
Goat anti-Rabbit IgG, Affinity Purified	A009-10MG/-25MG
Arbor Assays ISWE Plate Coating Course	Contact us for details

## OTHER MATERIALS REQUIRED

ISWE Mini-Kit: Testosterone, ISWE001; Cortisol, ISWE002; or Progesterone, ISWE003.

Distilled or deionized water.

Repeater pipet capable of dispensing 25, 50 and 100  $\mu$ L.

Colorimetric reader capable of reading optical density at 450 nm.

## 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.

For antibodies and conjugates using a peroxidase-based readout system, buffers (including other manufacturers' Wash Buffer) containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure any plate washing system is rinsed well without deionized water prior to using supplied Wash Buffer.

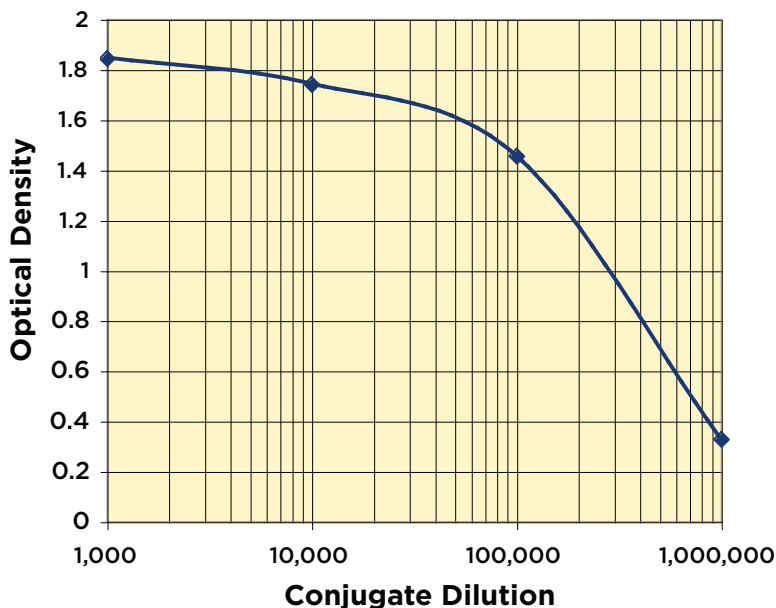
The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

## ASSAY DEVELOPMENT SUGGESTIONS

We recommend determining the appropriate dilutions of antibodies and conjugates for assays be done in a stepwise process. If using an Arbor Assays supplied ISWE Mini-Kit for cortisol, progesterone or testosterone dilute the Antibody Concentrate or Conjugate Concentrate 1:50 into the appropriate Assay Buffer or the Conjugate Diluent.

For other non-ISWE Mini-Kit antibodies and conjugates we suggest that you determine the proper titer of either the antibody and conjugate sequentially.

Here is a typical conjugate titer. Make a 1:100 dilution in the Conjugate Diluent, followed by further 1:10 dilutions.

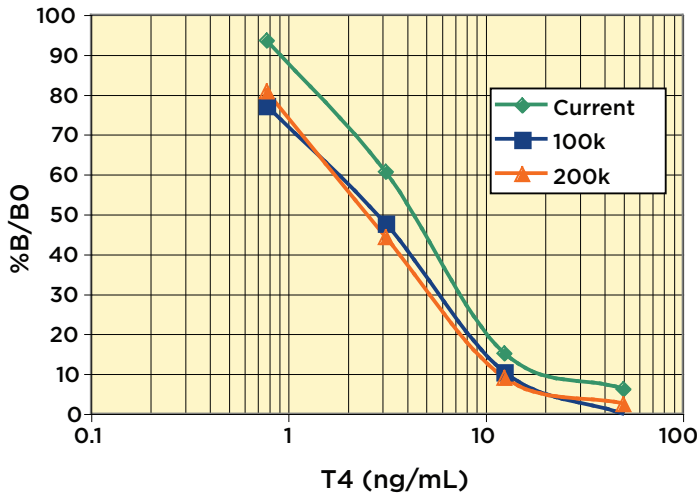


For this conjugate further dilutions in the range 1:100,000 to 1:400,000 may also be undertaken. Keep all other variables constant. Similarly using a non-ISWE Mini-Kit antibody the antibody would be diluted into the diluted 1X Assay Buffer (provided with stabilizers to allow 4°C storage of antibodies without degradation). After determining the optimum dilution of the conjugate (in this case about 1:200,000) the antibody is titered using the diluted conjugate in Conjugate Diluent.

## POST TITERING EVALUATIONS

After initial titrating of antibodies and conjugates we suggest preliminary standard curves are run to ensure the curve matches your analytical range for determinations. You may have to adjust these dilutions to ensure the optimum part of the standard curves (i.e. between 20% to 80% B/B0) is where your crucial sample concentrations will fall. Below is an abbreviated standard curve for thyroxine with 2 dilutions of a new conjugate.

A typical assay protocol uses 100  $\mu\text{L}$  of sample or standard, followed by 50  $\mu\text{L}$  of conjugate and 50  $\mu\text{L}$  of antibody in a 2 hour room temperature incubation with shaking.



## STORAGE OF ANTIBODIES, CONJUGATES, AND BUFFERS

Almost all antibodies you will use are stable in solution at 4°C and will not lose binding activity over their use. If diluted in a buffer with a preservative, such as our supplied Assay Buffer which contains Kathon as a preservative, they will be stable for many years.

Most steroid-HRP conjugates will be stable in solution if diluted in Conjugate Diluent and stored at 4°C for months. They may be stored in solution, either as stocks or diluted to working concentrations, indefinitely at -20°C.

Non-Arbor Assays Assay Buffers and Wash Buffer are also stable at 4°C if treated with 0.1% Kathon preservative.

## TROUBLESHOOTING

Area	Problem	Cause
Assay Performance	Variability in assay optical density	Uneven plate coating due to detergent washed labware
		Assay Components stored at inappropriate temperatures or humidities
		Use of ABTS substrate
	Decreasing assay optical density.	Plates are not desiccated properly due to failure to close ziplock or moisture interacting with silica gel desiccant.
Conjugate dilutions stored at inappropriate temperatures with or without conjugate stabilizers.		

### CONTACT US

We welcome your questions and comments regarding our products.

Tel: 0032 16 58 90 45

Email: [info@gentaur.com](mailto:info@gentaur.com)

## 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 US

**We welcome your questions and comments regarding our products.**

**Tel: 0032 16 58 90 45**

**Email: [info@gentaur.com](mailto:info@gentaur.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.

*DetectX<sup>®</sup>, ThioStar<sup>®</sup> and the Arbor Assays logo are all registered trademarks.*

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