



ARBOR ASSAYS™  
Interactive Assay Solutions™



**ISWE**

**Donkey anti-Sheep IgG  
Plate Coating Kit (10 Plate)**

Catalog Number ISWE009

Official Supplier  
to ISWE



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



## 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.
Donkey anti-Sheep IgG Coated Plate	X061-1EA
Assay Buffer 5x Concentrate	X065-110ML
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
Coating Buffer 20x Concentrate	X108-100ML
Blocking Buffer 10x Concentrate	X109-250ML
Donkey anti-Sheep IgG, Affinity Purified	A010-10MG/-25MG/-50MG/-100MG
Goat anti-Mouse IgG Plate Coating Kit (10 Plate)	ISWE004
Goat anti-Rabbit IgG Plate Coating Kit (10 Plate)	ISWE005
ISWE Assay Development Kit	ISWE006
Arbor Assays ISWE Plate Coating Course	Contact us for details

## OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet capable of dispensing 100 µL or 250 µL.

Beakers capable of holding 100, 250, and 500 mL.

Magnetic stir bars.

Desiccator with Indicating Silica Gel.

## 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 plates need to be stored desiccated. The silica gel pack included in the foil ziplock bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziplock has not been closed properly.

## CAUTIONS

We strongly recommend dedicating one beaker and stir bar for each solution to be coated onto plates, such as a beaker and stir bar for coating Goat anti-Mouse IgG, a beaker and stir bar for coating Goat anti-Rabbit IgG, a beaker and stir bar for coating Donkey anti-Sheep IgG, and a beaker and stir bar for blocking buffer. These beakers and associated stir bars should be kept separate and should only be thoroughly rinsed with deionized water after use. **They should never be washed with detergent.** After washing the beaker and stir bar they should be allowed to air dry and be covered with Parafilm® or Clingfilm®.

Coated plates need to be dried after being coated and blocked. A desiccator with activated indicating silica gel should be used. The silica gel can be reactivated after use by heating to 120°C for >2 hours. Indicating silica gel will turn from blue, when it is dry, to pink when hydrated. Store activated desiccant in an air-tight container.

## CALCULATIONS

To coat a plate with antibody at 150 µL per well requires 14.4 mL of coating solution. We would suggest making an excess of 10% of the total volume of antibody needed to allow for priming and washing any tubing or tips. At 150 µL per well this would mean that 15.8 mL of coating solution should be made per plate.

To coat a plate with antibody at 250 µL per well requires 24 mL of coating solution and the 10% excess needed brings the coating solution volume to be about 26-27 mL per plate.

We suggest blocking at 350 µL per well and one 96 well plate will require 33.6 mL of block per plate. The 10% excess that may be needed to allow for priming and washing any tubing or tips brings this volume to 36-37 mL per plate.

We suggest using the coating antibodies at 10 µg/mL to allow even low titered antibodies to be effectively captured on the plate. To coat a plate at 150 µL per well requires 0.158 mg of antibody for 15.8 mL of coating solution. To coat a plate at 250 µL per well requires 0.264 mg of antibody for 26.4 mL of coating solution.

## PLATE COATING PROCEDURE

1. Make sure the area is clean and free of dust or particulate material.
2. Calculate the amount of coating solution needed to coat the desired number of plates.
3. Prepare the diluted coating buffer and remove the volume of antibody to be spiked into the buffer. Spike the coating buffer with the required amount of antibody, stirring thoroughly.
4. Pipet the required volume of coating solution into each well. For smaller number of plates we recommend a Cornwall type syringe fitted with a 8-channel needle, such as those made by BD or V&P Scientific.
5. Stack the plates on top of each other after coating, covering the top plate, and allow to sit at room temperature overnight.
6. The following day prepare the blocking buffer.
7. Aspirate the coating solution and refill each well with 350  $\mu$ L of blocking buffer. Stack the plates on top of each other after coating, covering the top plate, and allow to sit at room temperature overnight.
8. The following day aspirate the wells and place the plates in the desiccator at room temperature. The plates may take up to 3 days to dry completely.
9. Once the plates are dry, place 5 plates at a time in the ziplock plate bag with 2 desiccant packs and seal. The plates can be stored at 4°C for up to 2 years as long as they are desiccated.
10. Prior to using the plates, allow the ziplock bag containing the coated plate to come to room temperature before opening the ziplock bag. Any unused wells can be stored in the desiccated ziplock bag tightly closed for further use.

## TROUBLESHOOTING

Area	Problem	Cause
Plate Coating	Bubbles in wells	Blocked repeater, 8- or 12-channel pipet tip.
Assay Performance	Variability in assay optical density.	Uneven plate coating due to detergent in washed labware.
		Uneven volumes used for coating or blocking. Visual check of volumes used. Clean or replace tips on pipets.
	Decreasing assay optical density.	Plates are not desiccated properly due to failure to close ziplock or moisture interacting with silica gel desiccant.

### 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

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**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.

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