ARBOR ASSAYS™
Interactive Assay Solutions™

ISWE

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

Catalog Number ISWE009

Official Supplier to ISWE

INTERNATIONAL SOCIETY of WILDLIFE ENDOCRINOLOGY

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

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WEB ISWE009 240923

PRINCIPLES OF PLATE COATING

This kit was designed to help ISWE scientists coat secondary antibodies onto plates. The kit provides all of the materials needed to coat a secondary antibody onto a microtiter plate, block the plate to reduce non-specific binding (NSB), and store the dried plate. This kit, plus our Assay Development Accessory kit, Catalog Number ISWE006, provide you with the best possible tools for developing a stable, reproducible secondary antibody coated plate assay for a steroid or other molecule.

THEORY

The attachment of antibodies (and other proteins) to a plate is dependent on both electrostatic and hydrophobic binding of the antibody (Ab) to the plastic surface. Antibodies will bind to almost any chemically or non-chemically modified surface, i.e. glass, plastic, metal, etc., and assays can be developed on any of these solid phases. For most biological applications, the attachment of a secondary Ab is carried out onto microtiter plates. The binding of a pure secondary antibody, such as affinity-purified donkey antibody to sheep IgG (DxS IgG) or affinity-purified goat antibody to rabbit IgG (GxR IgG) or to mouse IgG (GxM IgG) is carried out at the pI of the antibody where it has no net charge. The Ab has low aqueous solubility at this pH. The pI for most polyclonal antibodies is between pH 7 and 8.

The supplied Coating Buffer is at pH 8.0. Our Coating Buffer is supplied as 10mM sodium phosphate with preservative added. We recommend using the DxS IgG, GxR IgG, or GxM IgG antibodies at 10 µg/mL. The antibody should be diluted in the Coating Buffer in a new polyethylene beaker with a new Telon stir bar. The beaker and stir bar should be designated to be used only for coating one antibody and not be used for any other purposes. The beaker and stir bar should be washed with DI water **only** and allowed to air dry. It should not be washed with detergent. We suggest coating at 150 µL/well if the final well reaction volume is 100 µL or 250 µL/well if your immunoassay reaction is 200 µL.

SUPPLIED COMPONENTS

Donkey anti-Sheep IgG Antibody

An affinity-purified donkey antibody specific for sheep IgG heavy and light chains diluted in a buffer with preservatives.

2 mg — Catalog Number A010-2MG

Coating Buffer Concentrate

A 20X Concentrate of Coating Buffer in a special stabilizing solution.

10 mL — Catalog Number X108-10ML

Blocking Buffer Concentrate

A 10X Concentrate of Blocking Buffer in a special stabilizing solution.

25 mL 2 Each — Catalog Number X109-25ML

Strip-Well Microtiter Plates

Corning Costar High Binding strip well plates.

2 Bags of 5 Each — Catalog Number X139-5EA

5-Plate Bag with Desiccant

Metallic-laminated plate bag with ziplock closure complete with 2 indicating desiccants per bag.

2 Each — Catalog Number X140-1EA

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
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

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 µL 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. The measured humidity in the desiccator should be <20% before packaing plates.
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.

ADDITIONAL RESOURCES

Please review information on our website. Go to www.ArborAssays.com/resources and view the Video section for helpful information on perfecting assays.

Also available in the Resource section are protocols for performing extractions on various sample types and handling saliva samples. There are also links to web sites we use for gathering information on chemicals, reference values for different antigens, second hand reconditioned or refurbished assay instruments and a host of other information.

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

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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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