

# Estrogen (E1/E2/E3) Plate, Detailed ELISA Procedure

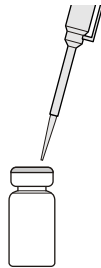
## 1. Sample Pretreatment

Filter the samples if necessary and add methanol to obtain a final methanol concentration of 10% (v/v)



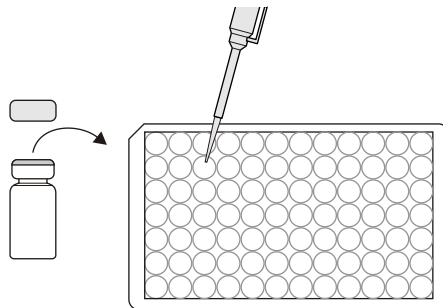
## 2. Reconstitution of Antigen-enzyme Conjugate

Reconstitute antigen-enzyme conjugate powder with 7mL of buffer solution. Mix by filling tip and expelling the contents with a pipette.



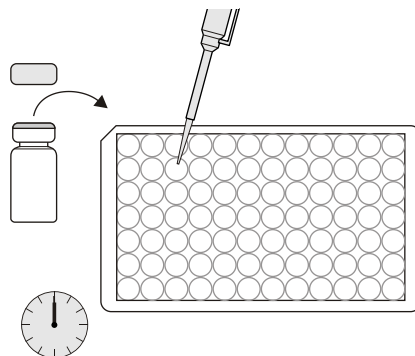
## 3. Addition of Standard/Sample and Conjugate

Add 100uL of E2 standards (or sample) and 100uL of conjugate solution in each **uncoated** well of a microplate. We recommend using duplicates or triplicates. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents.



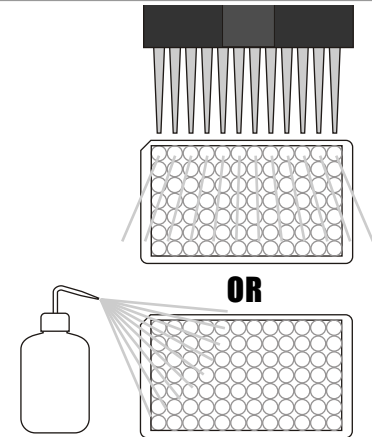
## 4. Incubation (Competitive Reaction)

Dispense aliquots of 100uL of the above mixture into each **antibody coated** well of a microplate. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate for 60 minutes at room temperature.



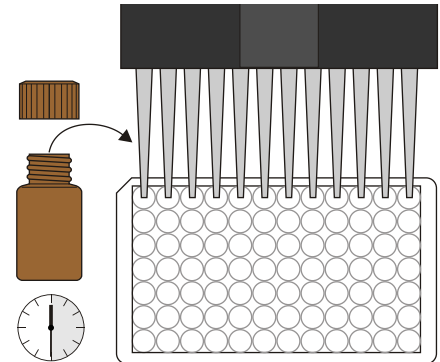
## 5. Washing Solution

Dilute 6-fold wash solution with 5 volumes of distilled water. Rise each microplate well with 300uL of the 1x wash solution and repeat 3 times.



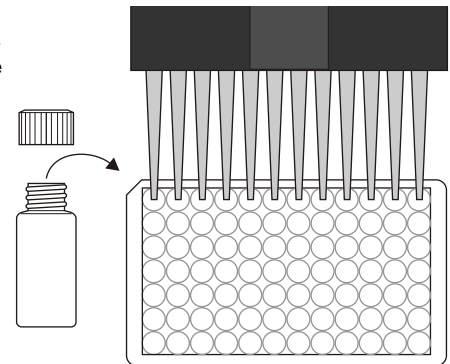
## 6. Addition of Color Solution

Dispense 100uL of the Color Solution into each well successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate for 30 minutes at room temperature.



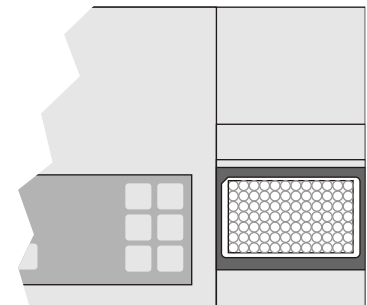
## 7. Addition of Stopping Solution

Add 100uL of stop solution to the wells in the same sequence as for the substrate solution using a multi-channel pipette or a stepping pipette.



## 8. Quantification

Measure the absorbance at 450nm for each standard solution and generate a standard curve. Calculate the quantity of ES in a sample from the absorbance reading.



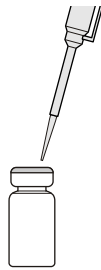
For Ordering or Technical Assistance Contact:

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# Estrogen (E1/E2/E3) Plate, Concise ELISA Procedure

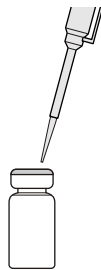
## 1. Sample Pretreatment

Filter the samples if necessary and add methanol.



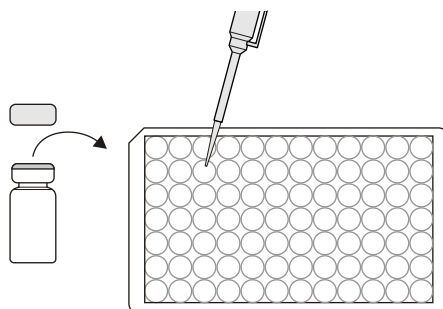
## 2. Reconstitution of Antigen-enzyme Conjugate

Reconstitute conjugate bottle with 7mL of buffer solution.



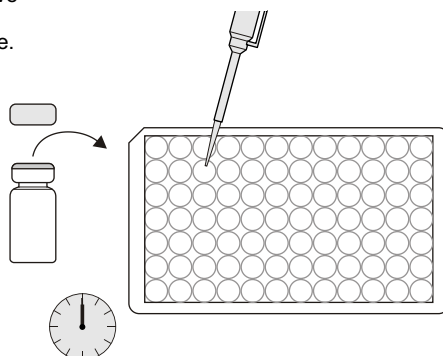
## 3. Addition of Standard/Sample and Conjugate

Add in duplicates 100uL of E2 standards (or sample) and 100uL of conjugate solution to each well of an **uncoated** microplate. Swirl to mix.



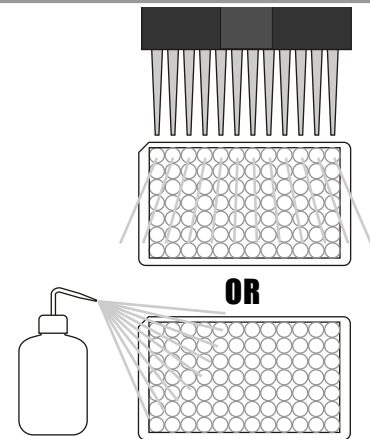
## 4. Incubation (Competitive Reaction)

Dispense 100uL of the above mixture into each well of an **antibody coated** microplate. Incubate for 60 minutes.



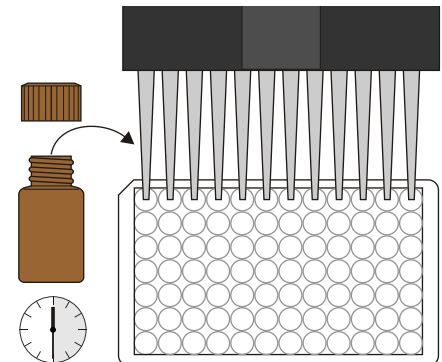
## 5. Washing Solution

Rise with 300uL of the 1x wash solution and repeat 3 times.



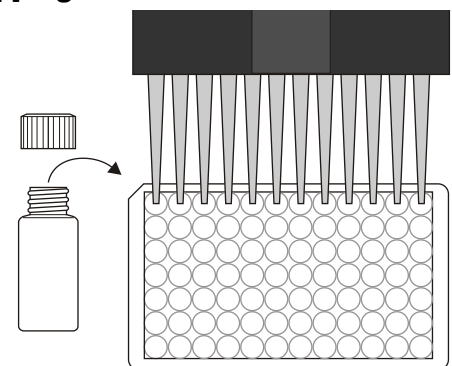
## 6. Addition of Color Solution

Add 100uL of the Color Solution into each well. Incubate for 30 minutes away from sunlight.



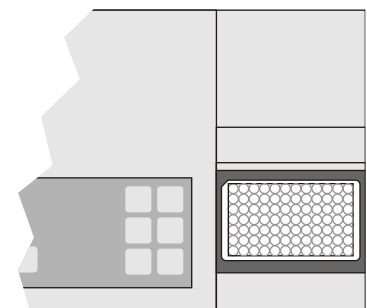
## 7. Addition of Stopping Solution

Add 100uL of stop solution into each well.



## 8. Quantification

Measure the absorbance at 450nm.



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