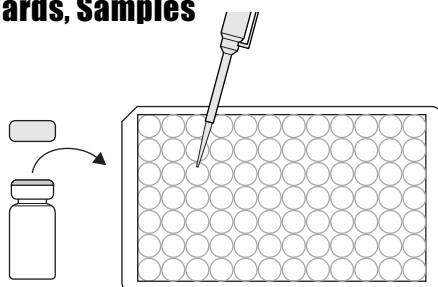


# Anatoxin-a Plate, Detailed ELISA Procedure

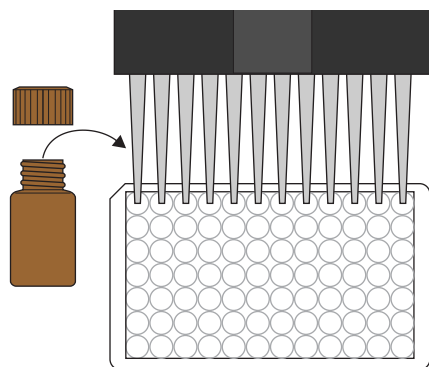
## 1. Addition of Standards, Samples

Add 50  $\mu$ l of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



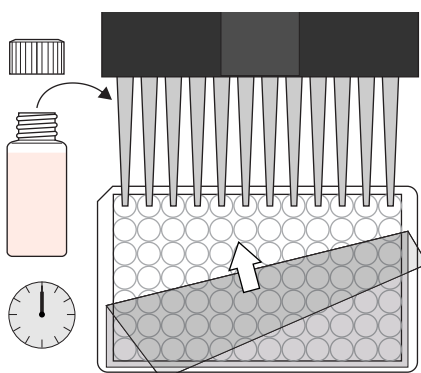
## 2. Addition of Enzyme Conjugate

Add 50  $\mu$ l of the enzyme conjugate to the individual wells successively using a multi-channel pipette.



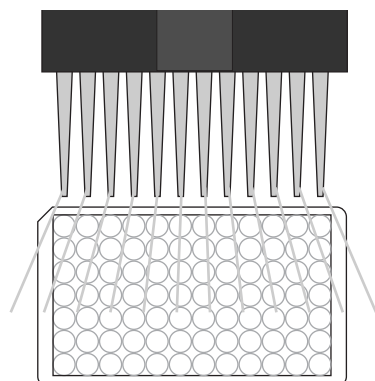
## 3. Addition of Antibody Solution

Add 50  $\mu$ l of the antibody solution to the individual wells 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. Be careful not to spill contents. Incubate the strips for 60 min at room temperature.



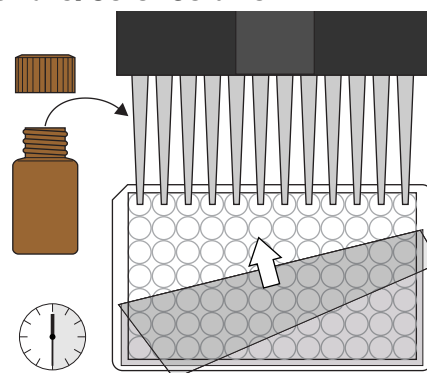
## 4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips four times with a multi-channel pipette. Please use at least a volume of 250  $\mu$ l of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



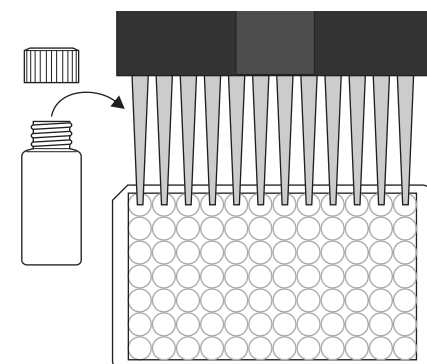
## 5. Addition of Substrate/Color Solution

Add 100  $\mu$ l of substrate/color solution to the individual wells 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. Be careful not to spill contents. Incubate the strips for 20-30 min at room temperature.



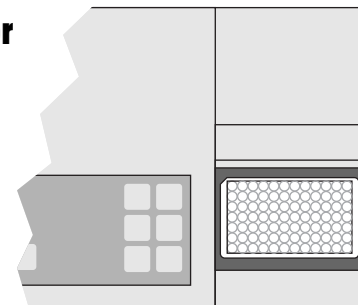
## 6. Addition of Stopping Solution

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



## 7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

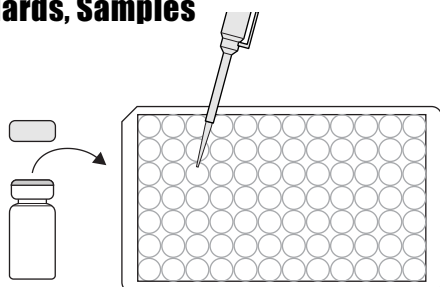


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# Anatoxin-a Plate, Concise ELISA Procedure

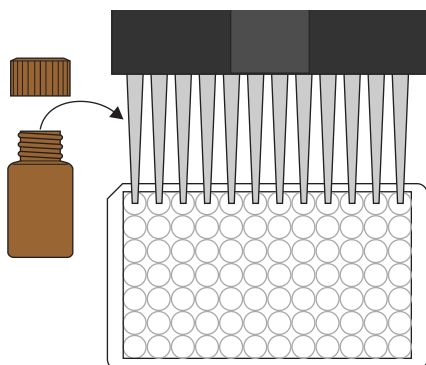
## 1. Addition of Standards, Samples

Add 50  $\mu$ L of standard solutions, control or samples.



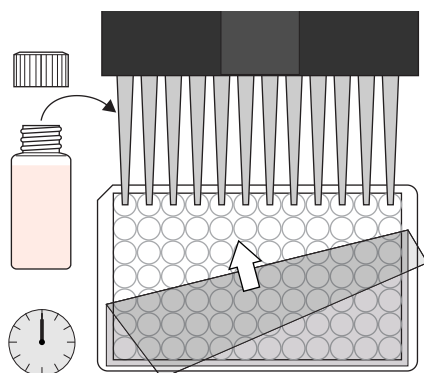
## 2. Addition of Enzyme Conjugate

Add 50  $\mu$ L of the enzyme conjugate.



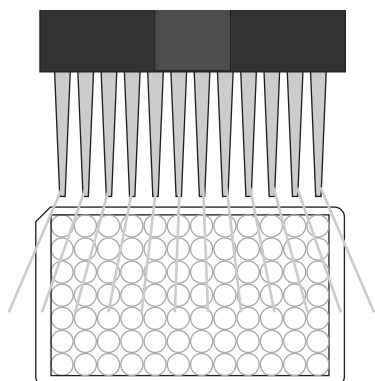
## 3. Addition of Antibody Solution

Add 50  $\mu$ L of antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 60 minutes at room temperature.



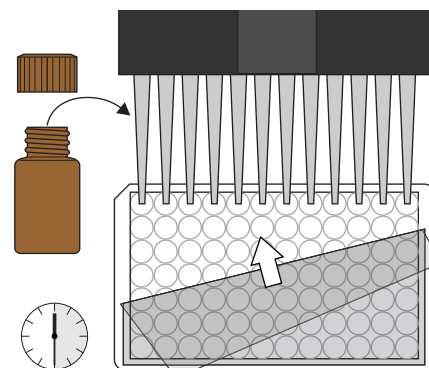
## 4. Washing of Plates

Wash the plates four times with 250  $\mu$ L of diluted 1X washing buffer.



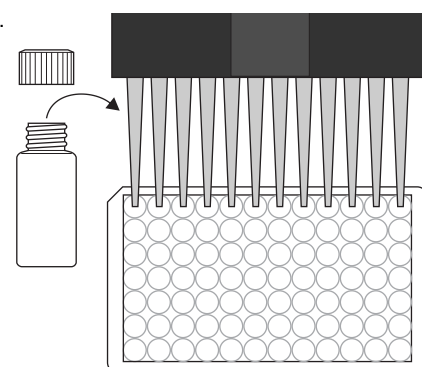
## 5. Addition of Substrate/Color Solution

Add 100  $\mu$ L of substrate/color solution. Incubate 20-30 minutes at room temperature and away from direct sunlight.



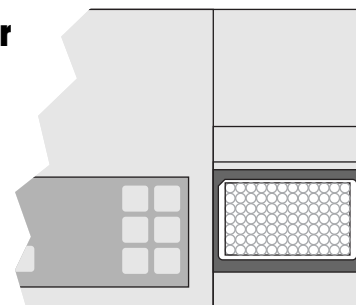
## 6. Addition of Stopping Solution

Add 100  $\mu$ L of stop solution.



## 7. Measurement of Color

Measure color at 450 nm. Calculate results.



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