

### 1. Addition of Standards and Samples

Add 125  $\mu$ L of each of the standards and samples into an individual glass test tube.



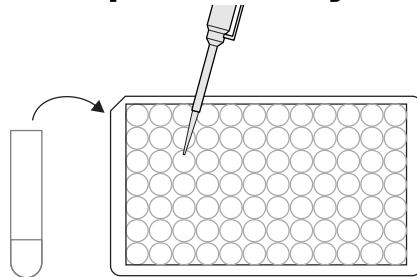
### 2. Addition of Antibody Solution

Add 125  $\mu$ L of each of the standards and samples into an individual glass test tube.  
Incubate 60 minutes at room temperature and away from direct sunlight.



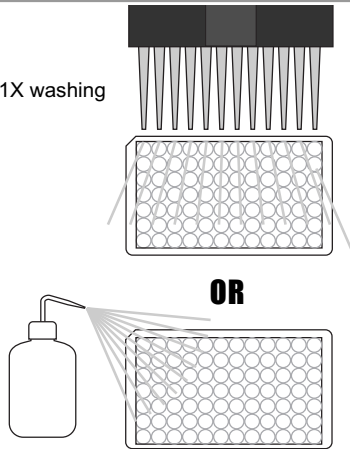
### 3. Addition of Standards/Samples with Antibody Solution (from step 2)

Add 100  $\mu$ L of the standards and samples.  
Incubate 60 minutes at room temperature and away from direct sunlight.



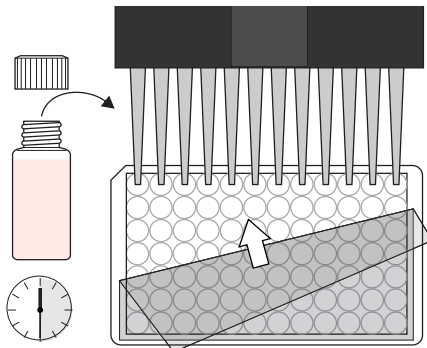
### 4. Washing of Plates

Wash the plates three times with 1X washing buffer. Fill to overflow.



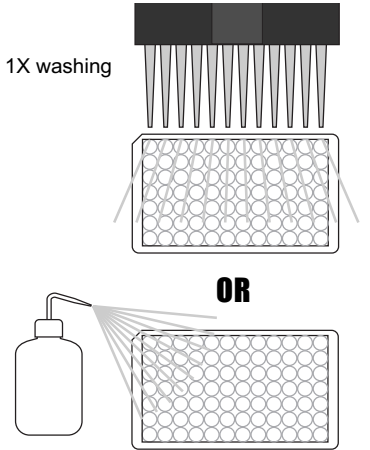
### 5. Addition of Anti-Rabbit Enzyme Conjugate

Add 100  $\mu$ L of the Anti-Rabbit enzyme conjugate. Cover and mix for 30 seconds by rotating on benchtop.  
Incubate for 30 minutes at room temperature.



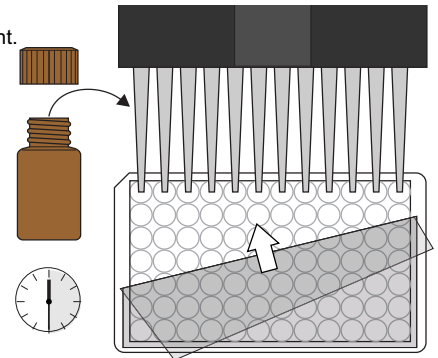
### 6. Washing of Plates

Wash the plates three times with 1X washing buffer. Fill to overflow.



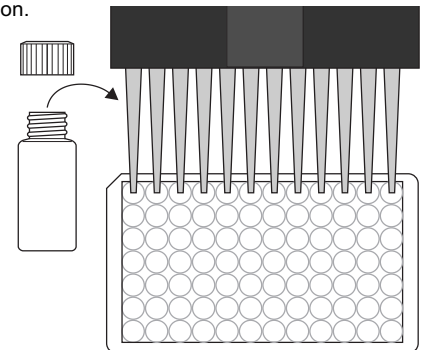
### 7. Addition of Color Solution

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



### 8. Addition of Stopping Solution

Add 100  $\mu$ L of stop solution.



### 9. Measurement of Color

Measure color at 450 nm.  
Calculate results.

