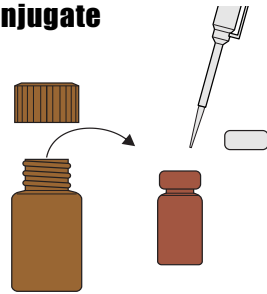


1. Reconstitute Enzyme Conjugate

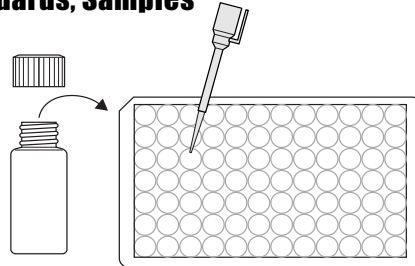
Reconstitute lyophilized enzyme conjugate with 2.0 mL Acrylamide conjugate diluent. Allow to sit for 5 minutes.

Note: One vial of conjugate is enough for approximately 40 wells. If more than one vial is needed mix reconstituted conjugates together before adding to plate array.



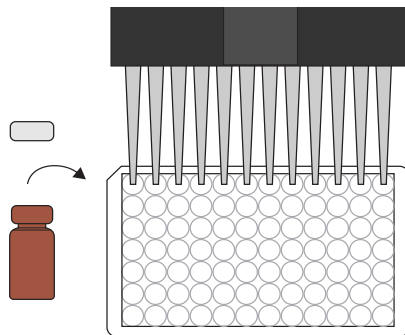
2. Addition of Standards, Samples

Add 50 uL of the **derivatized** standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



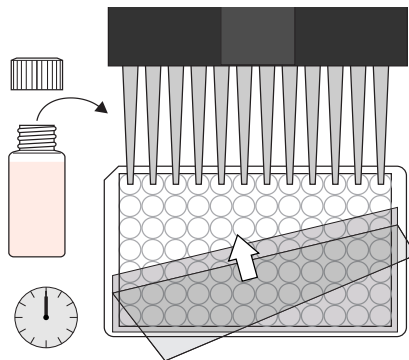
3. Addition of Enzyme Conjugate

Add 50 uL of the reconstituted enzyme conjugate to the individual wells successively using a multi-channel pipette or a stepping pipette.



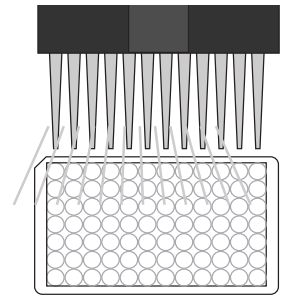
4. Addition of Antibody Solution

Add 50 uL of the Acrylamide antibody solution to the individual wells successively using a multi-channel or 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 refrigerated temperature (2-8°C).



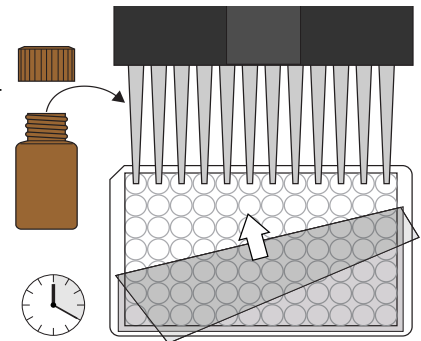
5. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink and blot. Wash the strips three times with a multi-channel pipette or wash bottle using the diluted 1X wash buffer. Please use at least a volume of 250 uL of diluted wash buffer for each well and each washing step. Blot inverted plate after each wash step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



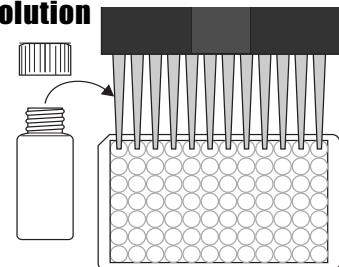
6. Addition of Substrate/Color Solution

Add 150 uL 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 min at room temperature away from direct sunlight.



7. Addition of Stopping Solution

Add 100 uL 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. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader within 15 min. Calculate results.

