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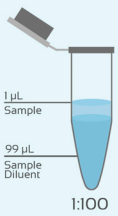
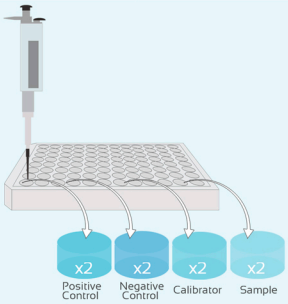
Step 1



Sample Preparation

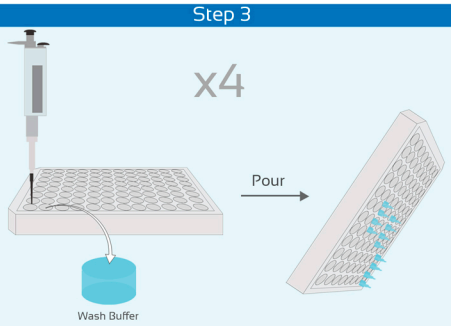
Step 2



Add 100 µl of the positive control, the negative control, the calibrator and the prepared sample into the individual wells of the microplate (2x wells each solution).

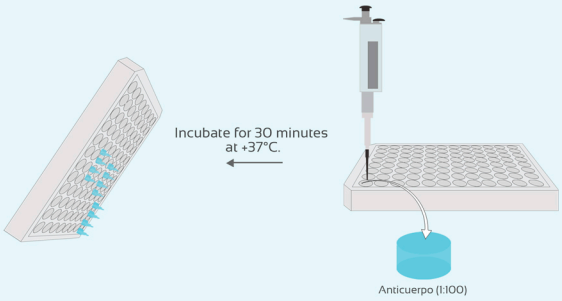
Incubate for 60 minutes at +37°C.

Step 3



Empty the wells and then wash 4 times using 300 µl of 1X wash buffer in each wash. After washing, completely remove all liquid from the microplate by tapping it on absorbent paper.

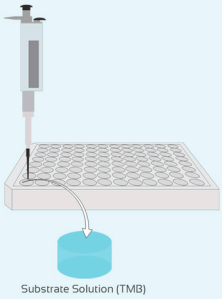
Step 4



Add 100 µl of the dilution (1:100) of the HRP-conjugated antibody to the wells. Incubate for 30 minutes at +37°C.

Repeat Step 3 (x4)

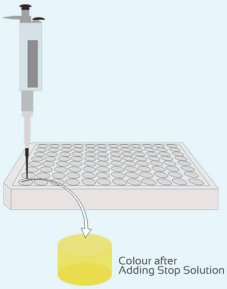
Step 6



Add 100 µl of the substrate solution (TMB) to each well of the microplate.

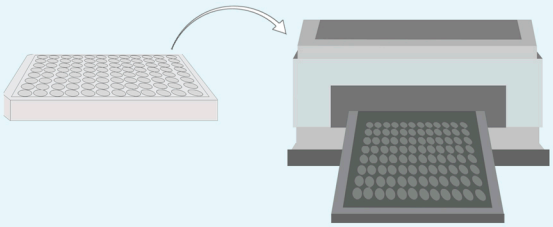
Incubate 10 minutes at room temperature (+18°C and +24°C) and protected from light.

Step 7



Add 100 µl of the stop solution (1X) to each well.

Absorbance measurement



Measure the optical densities (OD) of each well in a microplate spectrophotometer at 450 nm

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