

Test Procedure

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| 1 | Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used. |
| 2 | <p>QUALITATIVE ELISA TEST FORMAT</p> <p>Pipette 10 µL of ready-to use Negative Control, Reactive Control, and Samples into the respective wells of microtiter plate.</p> <p>Wells</p> <p>A1: Negative Control</p> <p>B1: Negative Control</p> <p>C1: Reactive Control</p> <p>D1 and on: Sample (Serum/Plasma)</p> |
| 3 | Cover the plate with adhesive foil. Incubate 60 min at room temperature (18-25°C). |
| 4 | Remove adhesive foil. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel. |
| 5 | Pipette 100 µL of ready-to use Peroxidase into each well. |
| 6 | Cover the plate with adhesive foil. Incubate 60 min at room temperature (18-25°C). |
| 7 | Remove adhesive foil. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel. |
| 8 | Pipette 100 µL of TMB Substrate Solution into each well. |
| 9 | Incubate 20min (without adhesive foil.) at room temperature (18-25°C) in the dark. |
| 10 | Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow. |
| 11 | Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution. |

Interpretation of Results

For the run to be valid, the OD_{450/650} nm of Positive Control should be ≥ 1.00 and the OD_{450/650} nm of each Negative Control should be < 0.200 . If not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD_{450/650} nm of the negative controls by 3.

I.e.;

If "Sample OD_{450/650} the mean OD_{450/650} of Negative Controls" is ≥ 3 , the sample is POSITIVE

If "Sample OD_{450/650} the mean OD_{450/650} of Negative Controls" is < 3 , the sample is NEGATIVE

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