

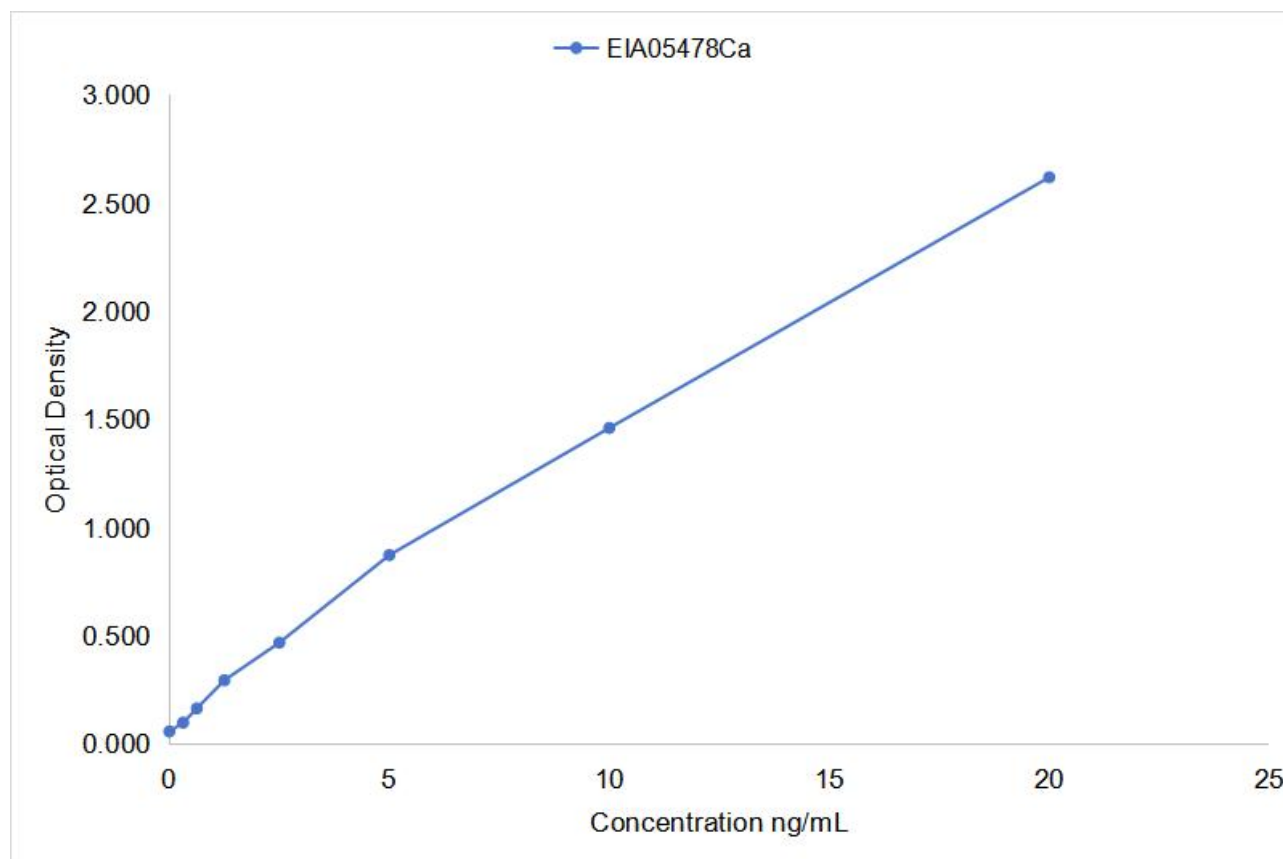




the dilution factor when calculating the final concentration.

Note: This protocol is for reference only. The concentration calculation of test samples must be based on the standard curve generated during the same experiment.

Reference curve



Note: Again, please note that this chart is for reference only, and that user sample concentrations should be based on the standard curve generated in the same assay run on the same microplate.

Summary of operating procedures

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1	Prepare reagents, samples and standards.
2	Add the prepared samples and standards to their respective wells and seal the plate. Incubate at 37°C for 90 minutes.
3	Wash the plate 2 times. Add the Biotinylated Antibody solution and seal the plate. Incubate at 37°C for 60 minutes.
4	Wash the plate 3 times. Add the prepared Enzyme Conjugate working solution and seal the plate. Incubate at 37°C for 30 minutes.
5	Wash the plate 5 times. Add the TMB Substrate Solution and incubate at 37°C in the dark for 10–30 minutes until a clear color gradient appears.
6	Add Color Reagent C to each well to terminate the reaction.
7	Use a microplate reader to measure the OD value at 450 nm within 10 minutes of adding Color Reagent C.

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Calculate the concentration of the test samples.