ELISA KIT TO DOSE CORTISOL IN SALIVA

Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with Cortisol. During the reaction, Cortisol in the sample or standard competes with a fixed amount of Cortisol on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to Cortisol. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution i s added to each well.

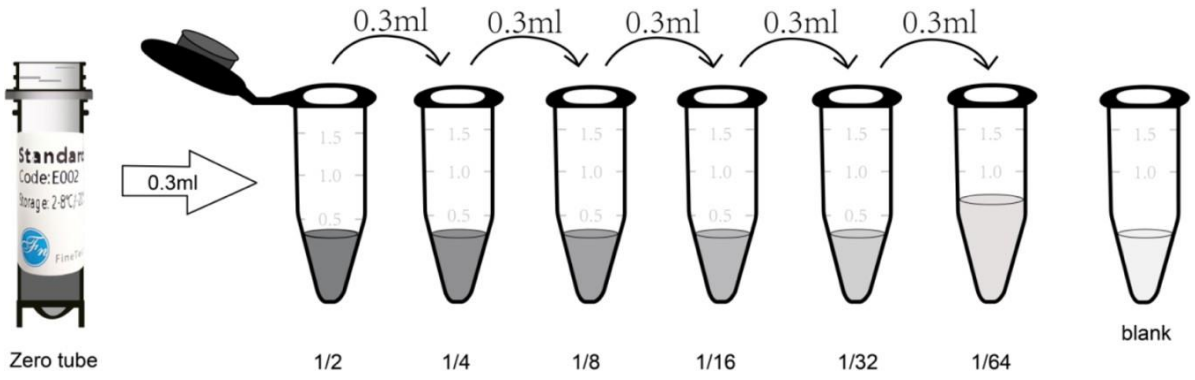
The enzyme-substrate reaction is terminated by the addition of a acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of Cortisol in the samples is then determined by comparing the OD of the samples to the standard curve. The concentration of the target substance was inversely proportional to the OD450 value.

Protocol

1. Wash the wells 1 time with 20µL of wash buffer
2. Add 50µL of standard* or sample and immediately add 50µL of biotin-labeled working solution (biotin). Incubate for 5 minutes at RT
3. Wash the wells twice with 20µL of wash buffer
4. Add 100µL of HRP-streptoavidin coniugate working solution (SABC) and incubate for 5 minutes at RT
5. Wash the wells twice with 20µL of wash buffer
6. Add 33µL dilution buffer
7. Add 30µL of the HRP substrate (TMB) and incubate for 5 minutes at RT
8. Add 30µL of stopping solution and read immediately the absorbance at 450nm with spectrophotometer

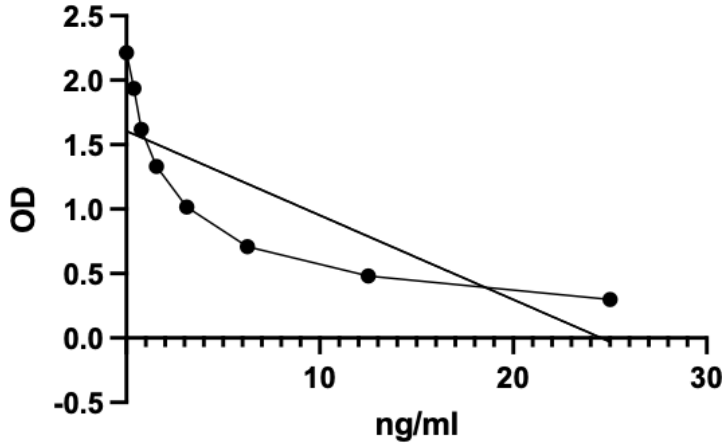
How to prepare standard solutions for the standard curve

Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.562ng/ml, 0.781ng/ml, 0.391ng/ml, 0ng/ml. The curve is constituted by 8 serial point dilutions (From tube 1 (with the highest concentration of cortisol to tube 8 with the lowest concentration of cortisol).



STD. (ng/ml)	N.
0	8
0.391	7
0.781	6
1.562	5
3.125	4
6.25	3
12.5	2
25	1

Results obtained from the practical activity



Four parameters
logistic curve (4PL)
R2=0.99

The average value for the points of the standard curve were:

Standard tube number	Average OD	ng/mL
1	0.298	25
2	0.482	12.5
3	0.708	6.25
4	1.015	3.13
5	1.332	1.56
6	1.618	0.781
7	1.936	0.391
8	2.214	0

The average values for the tested samples were:

Sample name	Average OD	ng/mL
Saliva sample – morning	0.59	8
Saliva sample – evening	0.88	4