The specific binding of AFP to AP-273

1. Coated: take 20μL (10mg / ml) Purified protein(AFP), then take 1980μL coated buffer, 100μL per well to add in 96-wells plate, Incubate in a 37 ℃ incubator for 2 hours.
2. Emptied and dry the residual liquid, and washed the 96-wells plate twice with 300μl of washing buffer.
3. Added 300μL (5 mg / ml) of BSA block Buffers to each well and allowed to stand at 4℃ for 1 hour
4. Discard the liquid, wash the plate with 300μl washing-buffer 3 times, during the last time after washing empty the liquid and pat dry
5. The aptamer was arranged into diluted solution of 0.2,0.4,0.6,0.8,1.0μmol/L in concentration, 100μl of the appropriate concentration of the aptamer was added to 96-wells .The blank group was added with TE buffer, and the above substances were incubated at 37 ℃for 2h.
6. Discard the liquid, wash the plate with 300μl washing-buffer 3 times, during the last time after washing empty the liquid and pat dry.
7. Added 100μl 1: 2000 diluted HRP- streptavidin to the 96-wellss and incubated at 37 ° C for 1.5 h.
8. discard the liquid，wash the plate with 300 μl of washing buffer three times, then soak with 300μl of rinse buffer for 5 min, then empty the liquid and pat dry，and then wash the plate with 300μl washing buffer 2 times，during the last time after washing empty the liquid and pat dry.
9. Added 10μL of TMB solution to 96-wells. After 20 mins of color development in the dark, added 50μL stop solution to terminate the reaction immediately. After the reaction was stopped, read the absorbance value at 450 nm.