

ELISA(Enzyme-Linked Immunosorbent Assay)

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1. Antigen is put in coating buffer(A+B) - adjusted 0.1ug/ml - 100ug in each well in cold room overnight

A:0.168g NaHCO₃/100ml H₂O

B:0.212g Na₂CO₃(anhydron)/100ml H₂O

68ml(A) + 32ml(B) PH 9.6

2. Block for 1 hr in 0.1M CMFPBS + 0.1%Tween20 + 1%BSA

3. Rinsed with 0.1M CMFPBS + 0.1%Tween20 a few minutes 5 times

4. Incubate in 1st ab for 1.5 hrs at r.t.

5. Rinsed with CMFPBS+0.1%Tri20 5 times

6. Incubate in 2nd ab for 1.5 hrs at r.t.

7. Rinsed with CMFPBS+0.1%Tri20 5 times

8. Develop in 0.1%OPD and 0.01%H₂O₂ in phosphate/citrate buffer(A+B)

A:0.2M Na₂HPO₄(71.7g/1000ml)

B:0.1Mcitric acid(19.21g/1000ml)

25.7ml of A+24.3ml of B - diluted to a total 100ml

9. Stopped by addition of 2N H₂SO₄ 100ul/well