Sandwich ELISA

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Ziru Li

Sandwich ELISA protocol - need optimization

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1. Incubate plate with capture antibody in carbonate buffer overnight.
   - 100ul/well at 4° C; seal plate.
2. Wash 3 times with PBS.
   - 100ul/well
3. Block wells with PBS-1%M.
   - 100ul/well on shaker for 2 hours; seal plate.
4. Wash 3 times with PBS-T.
   - 100ul/well
5. Incubate wells with serial dilutions of antigen in PBS.
   - 100ul/well on shaker for 2 hours; seal plate.
6. Wash 3 times with PBS-T.
   - 100ul/well
7. Incubate wells with detection antibody in PBS-0.2%M.
   - 100ul/well on shaker for 1 hour; seal plate.
8. Wash 6 times with PBS-T.
   - 150ul/well on shaker 2min each.
9. Add substrate. [Always use a new trough for adding substrate]
   - Colorimetric (TMB): read $A_{370/655}$ on plate reader in kinetics mode right after substrate is added.
     Alternative ways:
     - Read endpoint absorbance after 45 minutes incubation on shaker.
     - Add stop solution (1M H_2SO_4) at 45 minutes and read absorbance at 450nm (optimization needed)
   - Chemiluminescent (Pierce): Mix reagent 1&2 in 1:1 ratio and read on plate reader after 5 minutes incubation.

Bicarbonate buffer: 1.59g Na2CO3, 2.93g NaHCO3, DI H2O to 1000mL, pH to 9.6
10xPBS: NaCl 80.0g, KCl 2g, Na2HPO4.7H2O 17.3g, KH2PO4 2g, DI H2O to 1000mL, pH to 7.2
PBS: 10xPBS 100mL, DI H2O 900mL, pH to 7.2

PBS-1%BSA: 1% BSA in PBS (e.g. 2g BSA in 200mL PBS)
PBS-T: 0.05% Tween20 in PBS-1%BSA (e.g. 250uL Tween20 in 500mL PBS-1%BSA)
PBS-0.2%BSA: 0.2% BSA in PBS (e.g. 0.2g BSA in 100mL PBS)
Or
(Remake the following every week)
PBS-1%M: 1% non-fat dry milk in PBS (e.g. 2g dry milk in 200mL PBS)
PBS-T: 0.05% Tween20 in PBS-1%M (e.g. 250uL Tween20 in 500mL PBS-1%M)
PBS-0.2%M: 0.2% non-fat dry milk in PBS (e.g. 0.2g dry milk in 100mL PBS)