Monoclonal Antibody Screening by Direct ELISA

Stock solutions:

PBS: 20 mM NaPi pH=7.5, 150 mM NaCl

PT: 0.1% Tween 20 in PBS

Blocking solution: 3% BSA in PT. Store at -20 C

Developing solution: 10 mg o-phenylenediamine in 25 ml of a buffer with pH=5.5 (say, 20 ml of H_2O + 5.16 ml of 0.5 M Na_2HPO_4 + 645 µl of 1.5 M citric acid) + 12 µl of 30% H_2O_2. This solution is light sensitive and should be prepared just before use.

1- Coat the plate wells with 50 µl of antigen (0.2 µg/ml in PBS). Incubate 1h at RT

2- Wash the plate (discard the well solution, add 200 µl of PT per well and wait about 2 min. Repeat this cycle another 2 times and discard the late well solution)

3- Block the plate wells with 150 µl of blocking solution. Incubate 1h at RT

4- Discard the blocking solution and add 50 µl of the hybridoma supernatants in different wells. Incubate 30 min at RT

5- Wash the plate

6- Add 50 µl of the peroxidase-coupled antimouseIgs antibody (1/5000 in blocking solution). Incubate 30 min at RT

7- Wash the plate

8- Incubate the plate wells with 100 µl of developing solution until color change is evident

9- Stop the reaction by adding 50 µl of 2.5 M H_2SO_4 per well

10- Measure the absorbance at 492 nm

NOTES:

- This is only a semiquantitative assay. If you want a quantitative assay, you have to dilute the primary antibody (step 4) at least 1/20 in blocking solution and probably much more. Anyway, direct ELISAs are adequate for quantitative assays only with pure samples. In any other case, you should use indirect ELISAs or sandwich ELISAs.

- Sodium azide is an inhibitor of peroxidase.