

## MEI Protocol

### ELISA Assay

XL Yang, Jan. 20, 2002

1. Determine the optimal reagent concentrations by criss-cross serial dilution analysis<sup>#</sup> (option)
2. Plate coating (Immulon 2, Dynatech #011-010-3450 or Immulon 4, Dynatech #011-010-3850)  
Use 100 µl/well of capture antibody (1-5 µg/ml) in coating buffer\* overnight at 4°C (sealed plates can be stored at 4°C with capture antibody for months).
3. Rinse plate 3 times with washing buffer\*\*.
4. Block with 200 µl/well of blocking buffer\*\*\* overnight at 4°C.
5. Rinse plate 3 times with washing buffer.
6. Sample loading  
Make a serial dilution of the standard antigen.  
Add 50 µl/well of samples and standard antigen to incubate for 2 hrs at RT (dilute the samples with blocking buffer).
7. Rinse plate 3 times with washing buffer.
8. Add 100 µl of biotinylated specific antibody (diluted in 1:1,000-5,000 in blocking buffer) for 2 hrs at RT.
9. Rinse plate 3 times with washing buffer.
10. Add 100 µl of avidin-conjugated HRP (1:1,000 in blocking buffer) for 1 hr at RT.
11. Rinse plate 3 times with washing buffer.

12. Develop with 100 µl of OPD (*o*-phenylenediamine dihydrochloride tablet sets, Sigma P9187) in dark place for 30 min at RT.
13. Read the optical density of the reaction under a wavelength of 450 nm.

\*Coating buffer

0.1 M sodium bicarbonate  
0.1 M sodium carbonate (pH 9.6)

\*\*Washing buffer

0.1% BSA  
1% Tween 20 in PBS

\*\*\*Blocking buffer

1% BSA  
1% Tween 20 in PBS

#Criss-cross serial dilution analysis to determine optimal reagent concentrations (refer to Current Protocols in Molecular Biology, 2001, pp11.2.16-17):  
Serial dilution titration analyses are performed to determine optimal concentration of reagents to be used in ELISAs. In this protocol, all three reactants in a three-step ELISA---a primary solid-phase coating reagent, a secondary reagent that binds the primary reagent, and an enzyme-conjugated tertiary developing reagent that binds to the secondary reagent---are serially diluted and analysed by criss-cross matrix analysis.

A. Prepare coating-reagent dilutions

1. Plate four 17x100 mm test tube in a rack and add 6 ml PBS to the last three tubes.  
In tube 1, prepare a 12 ml solution of coating reagent at 10 µg/ml in PBS.  
Transfer 6 ml of tube 1 to tube 2, and mix by pipetting up and down 5 times.  
Repeat this transfer and mix for tubes 3 and 4; the tubes now contain the coating reagent at 10, 5, 2.5 and 1.25µg/ml.

2. Dispense 50 $\mu$ l of the coating reagent solutions into wells of four Immulon microtiter plates (i.e., each plate is filled with one of the four dilutions). Incubate overnight at 37°C or 2hrs at RT.
3. Rinse plates 3 times with washing buffer and block plates with blocking buffer.

#### B. Prepare secondary-reagent dilutions

1. Place five 12x75 mm test tubes in a rack and add 3 ml blocking buffer to the last four tubes.

In tube 1, prepare a 4 ml solution of secondary reagent at 200 ng/ml in PBS.

Transfer 1 ml of tube 1 solution into tube 2. Pipet up and down 5 times. Repeat this transfer and mix for tubes 3 to 5; the tube now contain the secondary reactant at 200, 50, 12.5, 3.125 and 0.78 ng/ml.

2. Dispense 50  $\mu$ l of secondary reagent solutions into the first five columns of all four coated plates. The most dilute solution is dispensed into column 5, while solutions of increasing concentration are added successively into column 4, 3, 2 and 1. Thus, the 5<sup>th</sup> column contains 0.78 ng/ml and the first column 200 ng/ml. Incubate 2 hr at RT.
3. Wash plates 3 times with washing buffer..

#### C. Prepare developing-reagent dilutions

1. Place five 17x100 mm test tubes in a rack and add 3 ml blocking buffer to the last four tubes.

In tube 1, prepare a 6 ml solution of developing reagent at 500 ng/ml in blocking buffer.

Transfer 3 ml of tube 1 solution into tube 2 and mix. Repeat this transfer and mixing for tubes 3 and 4; the tube now contain the developing reactant at 500, 250, 125, 62.5 and 31.25 ng/ml.

2. Dispense 50  $\mu$ l of developing reagent solutions into the wells of rows 2 and 6 of each plate, dispensing the most dilute solution into row 6 and solutions of increasing concentration successively into row 5, 4, 3 and 2. Incubate 2 hr at RT.
3. Wash plates 3 times with washing buffer.

#### D. Measure

Add 75  $\mu$ l substrate to each well, incubate 1 hr at RT, and Read the optical density with a microtiter plate reader.