Competitive binding Protocol

The first experiment

1. Coating of Wells with Antibody

   100 µL of antibody diluted in buffer A is added to each well. The antibody should be directed against the antigen to be determined.

   Cover the plate with plastic film or aluminum foil and incubate at 4 °C overnight washing.

2. Wash the wells 3 times with buffer B. Alternatively, wash manually 3 times as follows: Empty the plate by inversion over a sink. Tap the inverted plate against some layers of soft paper tissue to remove residual liquid. Wash the plate by filling the wells by immersion in buffer B. Leave on the table for 3 minutes. Empty the plate as described above and repeat washing two more times.

3. Incubation with Test Samples (penicillin)

   100 µL of test sample (penicillin) or standard diluted in coating buffer is added per well.

   Cover the plate and incubate at 37°C for 2 hours.

   Wash as described in step 2. (Buffer B as washing buffer)

   100 µL of incubation buffer is added per well. Incubate at 37°C for 0.5h.

4. Adding test sample (penicillin)

   100 µL of test sample (penicillin) diluted in washing buffer is added to each one of the seven wells in each column, while 100 µL of pure PBS buffer is added to each one remaining well in each column. The concentration of test sample (penicillin) in each one of the eight wells is respectively 3000ppm, 1000ppm, 300ppm, 100ppm, 30ppm, 10ppm, 3ppm, oppm.

5. Addition of Florence-conjugated antibody (PBP-GFP)

   100 µL of Florence-conjugated antibody is added to each well in columns 1 to 3, 5 times, 25 times, 125 times diluted respectively. 100µL of supernatant (containing antibody) is added to each well in columns 4 to 6, 5 times, 15 times, 125 times diluted respectively.

   Incubate in 37°C for 0.5h.

6. Remove all the residual liquid.

7. Reading of Results

Read results directly through the black microwell plate using an automated or semi- automated photometer (plate-reader). Read the plate at 405 nm and 450nm. The subtraction of the absorbance at a wavelength of 509nm is recommended, but not essential.

The second experiment:

1. Coating of Wells with Antibody

   100 μL of antibody diluted in buffer A is added to each well. The antibody should be directed against the antigen to be determined.

   Cover the plate with plastic film or aluminum foil and incubate at 4 °C overnight

2. Washing.

   Wash the wells 3 times with buffer B. Alternatively, wash manually 3 times as follows: Empty the plate by inversion over a sink. Tap the inverted plate against some layers of soft paper tissue to remove residual liquid. Wash the plate by filling the wells by immersion in buffer B. Leave on the table for 3 minutes. Empty the plate as described above and repeat washing two more times.

3. Incubation with Test Samples (penicillin)

   100 μL of test sample (penicillin) or standard diluted in coating buffer is added per well.

   Cover the plate and incubate at 37°C for 2 hours.

   Wash as described in step 2. (Buffer B as washing buffer)

   100 μL of incubation buffer is added per well. Incubate at 37°C for 0.5h.

4. Adding test sample (penicillin)

100 μL of test sample (penicillin) diluted in washing buffer is added to each one of the seven wells in columns 1, 2, 4, 5, while 100 μL of pure PBS buffer is added to each one remaining well in each column. The concentration of test sample (penicillin) in each one of the eight wells is respectively 3000ppm, 1000ppm, 300ppm, 100ppm, 30ppm, 10ppm, 3ppm, 0ppm.

5. Addition of Florence-conjugated antibody (PBP-GFP)

   100μL of supernatant (containing antibody) 25 times diluted in PBS buffer is added to each well in column 1, 2, 4, 5.

   Incubate in 37°C for 0.5h.

6. Transport supernatant from the wells to the wells of another black microwell plate.

7. Reading of Results

Read results directly through the black microwell plate using an automated or semi- automated photometer (plate-reader). Read the plate at 405 nm and 450nm. The subtraction of the absorbance at a wavelength of 509nm is recommended, but not essential.

The third experiment：

1. Coating of Wells with Antibody

100 µL of antibody diluted in buffer A is added to each well. The antibody should be directed against the antigen to be determined.

Cover the plate with plastic film or aluminum foil and incubate at 4 °C overnight

2. Washing.

   Wash the wells 3 times with buffer B. Alternatively, wash manually 3 times as follows: Empty the plate by inversion over a sink. Tap the inverted plate against some layers of soft paper tissue to remove residual liquid. Wash the plate by filling the wells by immersion in buffer B. Leave on the table for 3 minutes. Empty the plate as described above and repeat washing two more times.

3. Incubation with Test Samples (penicillin)

   100 µL of test sample (penicillin) or standard diluted in coating buffer is added per well.

   Cover the plate and incubate at 37°C for 2 hours.

   Wash as described in step 2. (Buffer B as washing buffer)

   100 µL of incubation buffer is added per well. Incubate at 37°C for 0.5h.

4. Adding test sample (penicillin)

100 µL of test sample (penicillin) diluted in washing buffer is added to each one of the seven wells in columns 1, while 100 µL of pure PBS buffer is added to each one remaining well in each column. The concentration of test sample (penicillin) in each one of the eight wells is respectively 3000ppm, 1000ppm, 300ppm, 100ppm, 30ppm, 10ppm, 3ppm, 0ppm.

5. Addition of Florence-conjugated antibody (PBP-GFP)

   100µL of supernatant (containing antibody) 25 times diluted in PBS buffer is added to each well in column 1.

   Incubate in 37°C for 0.5h.

6. Transport supernatant from the wells to the wells of another black microwell plate.

7. Reading of Results

Read results directly through the black microwell plate using an automated or semi- automated photometer (plate-reader). Read the plate at 405 nm. The subtraction of the absorbance at a wavelength of 509nm is recommended, but not essential.

Reagents

A. Coating Buffer

0.01 M Phosphate Buffer, 0.15 M NaCl, pH 7.2

NaH_2PO_4, H_2O 0.35 g Na_2HPO_4, $2H_2O$ 1.34 g NaCl 8.47 g H_2O ad 1 litre - check pH (7.2 ± 0.2)

Store at 4 °C. Stable for 2 months. (12 mL of buffer A is needed for one microwell plate).

B. Washing Buffer/Dilution Buffer

0.01 M Phosphate Buffer, 0.50 M NaCl, 0.1% Tween 20, pH 7.2

NaH_2PO_4, H_2O 0.35 g Na_2HPO_4, $2H_2O$ 1.34 g NaCl 29.22 g Tween 20 (Merck, No. 822184) 1 mL

H_2O ad 1 litre - check pH (7.2 ± 0.2)

Store at 4 °C. Stable for 2 months. (1 to 3 litres of buffer B is needed for one microwell plate).