

Inhibition ELISA for Pneumococcal Capsular PS using Monoclonal Antibodies

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The inhibition ELISA can be used to detect and quantify capsular PS.

Note that the sensitivity of the assay is dependent on the concentration of polysaccharide used to coat the plates as well as the dilution of primary antibody used in the assay. The optimal concentration for each reagent should be determined empirically. The optimal coating concentration for PS typically ranges from 1 to 10 mg/L, and the optimal dilution of primary monoclonal antibody typically ranges from 50-fold to 10,000-fold.

Materials

- ELISA plates (medium-binding—we use Corning 9017)
- Pneumococcal capsular polysaccharide (purchased from ATCC)
- Phosphatase substrate (we use Sigma 1040)

Reagents

- Monoclonal antibody (culture supernatant or purified)
- Anti-mouse Ig-AP conjugate (Sigma A-0162)
- Test samples (pneumococcal culture lysates, or any sample with capsular PS)
- Standard (serially diluted capsular PS with known concentrations)
- Water (we use “Sterile Water for Irrigation, USP, from Hospira to prepare 10X PBS, 1X coating buffer, and 1X antibody buffer. Water from in-house purification systems, such as a Synergy system from Millipore, can be used for all other reagents)

Equipment

- Incubator (37C)
- ELISA plate reader with 405 nm filter
- Plate shaker

Solutions

3M NaOH

Use caution as preparation of this solution generates extreme heat and can be dangerous. Place 800 ml of water and a magnetic stir bar in a 2 L plastic beaker. Place the beaker on top of a magnetic stirrer. Weigh 120 gm of NaOH pellets and slowly add NaOH pellets to the water. If the water becomes too hot, stop adding the NaOH pellets and wait for it to cool. After adding all the NaOH pellets, mix the solution thoroughly, let it cool and bring the solution to 1 liter with water using a graduated cylinder. Mix well and store at room temperature for up to 12 months.

10 X PBS with sodium azide

Add 800 ml of **Hospira** water to a 2-liter beaker placed on a magnetic stirrer. Weigh out the dry chemicals listed below and add them to the water. Dissolve the chemicals and bring the volume to 1000 ml with **Hospira** water. Do not adjust pH (see instructions for preparing 1X coating buffer below). Sterilize the solution by filtering it with a 0.22 μm filter and store it in a sterile container at room temperature for up to 12 months.

Chemical	Weight (grams)
NaCl	80.00
KH ₂ PO ₄	3.14
Na ₂ HPO ₄ ·7H ₂ O	20.61
KCl	1.60
NaN ₃	2.00

1X Coating Buffer

Add 900 ml of sterile **Hospira** water to 100 ml 10X PBS with sodium azide prepared above. Check the pH of a small aliquot (do not insert the pH probe into the entire solution to avoid possible contamination). The pH should be 7.2 ± 0.2 . If the pH is not within this range, discard the solution, and prepare new 10X solution.

1X Antibody Buffer

Add 900 ml of sterile **Hospira** water to 100 ml 10X PBS with sodium azide prepared above. Add 0.5 ml Tween-20. Check the pH of a small aliquot (do not insert the pH probe into the entire solution to avoid possible contamination). The pH should be 7.2 ± 0.2 . If the pH is not within this range, discard the solution, and prepare new 10X solution.

10X Wash Buffer

Weigh out dry chemicals and dissolve in approximately 800 ml of water in 2 L plastic beaker. Mix the solution thoroughly using a magnetic stir plate and a stir bar. Add 33 ml of Brij-35 (30% w/v) and mix thoroughly. Add water to 1 Liter. Store at room temperature for up to 12 months.

Chemical	Weight (grams)
NaCl	80
KCl	1.60
Trizma Base	0.94
Trizma HCl	14.56

1X Wash buffer

Add 900 ml of water to 100 ml of 10X wash buffer. Mix thoroughly and check the pH. The pH should be 7.2 ± 0.2 .

Substrate Buffer

Calibrate the pH meter before making this solution because the pH of the substrate buffer is very important.

1. Place a 2000 ml plastic beaker with a magnetic stir bar on a magnetic stirrer in a fume hood.
2. Add about 800 ml of water.
3. Add 97 ml of diethanolamine to the water using a 100 ml graduated cylinder and mix the solution well with the magnetic stirrer.
4. Add 0.1 gm of MgCl₂·6H₂O to the solution.
5. While thoroughly mixing with a magnetic stirrer, adjust the pH to 9.8 ± 0.05 by slowly adding HCl.
6. Allow the solution to cool to room temperature.
7. Check the pH again and adjust to pH 9.8 ± 0.05 with HCl if necessary.

8. Add water to 1 L.
9. Since pH is critical, recheck the pH. If the pH is not 9.8 ± 0.05 , discard the diethanolamine buffer.
10. This buffer may be stored sealed in a light protected container at room temperature for up to 6 months.

ELISA Procedure

1. Dilute the PS of interest to the optimal coating concentration in 1X Coating Buffer.
2. Add 100 microliters of diluted PS to each well of ELISA plates and incubate for 5 hours in a 37°C humidified incubator.
3. After incubation, store coated plates at 4°C in a humidified chamber until needed. Plates can be stored for up to 1 month as long as they are prevented from drying.
4. Prepare serially dilutions of samples or standards in 1X Antibody Buffer. Sample dilutions may range from neat (undiluted) to 10,000-fold. Standard concentrations should range from ~50 mg/L to 0.01 mg/L. As noted above, the sensitivity of the assay is dependent upon the concentrations of the coating PS and primary antibody.
5. Wash previously coated plates 3 times with 1X Wash Buffer.
6. Add 50 microliters of diluted sample (or standard) to the appropriate wells. Make sure to include control wells that receive 1X Antibody Buffer instead of sample (or standard). These control wells will be used to calculate the maximum OD.
7. Add 50 microliters of diluted primary monoclonal antibody to the appropriate wells. Make sure to include control wells that receive 1X Antibody Buffer instead of primary monoclonal antibody. These control wells will be used to calculate background ODs.
8. Gently mix the plates at room temperature for 5 minutes using a plate shaker.
9. Incubate plates for 1 hour at 37°C.
10. Wash plates 3 times with 1X Wash Buffer.
11. Add 100 microliters of anti-mouse Ig-AP conjugate diluted in 1X Antibody Buffer (we generally use a 3000-fold dilution) to all wells.
12. Incubate plates for 1 hour at 37°C.
13. During the final 20 minutes of incubation, prepare substrate solution by adding substrate (1 mg/ml) to Substrate Buffer. Store at room temperature in the dark until needed.
14. Wash plates 3 times with 1X Wash Buffer.
15. Add 100 microliters of substrate solution to each well.
16. Incubate plates at room temperature until desired OD₄₀₅ is reached.
17. Stop the reaction by adding 50 microliters of 3 M NaOH solution to each well.
18. Read OD at 405 nm.
19. For PS quantitation, subtract the average background OD from all wells. Plot the normalized OD (OD of test sample/maximum OD) on the y-axis and the PS concentration on the x-axis (using a log scale). The PS concentration can be determined by comparing curves obtained with the standard and an unknown sample.

Reference

PE Bratcher et al. Microbiology 156:555, 2010.