Estimation of anti-OMV serum IgM level using indirect ELISA

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Method Article

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Abstract
This protocol is used for estimation of serum IgM level in mice using indirect ELISA.

Introduction
This method uses the estimation of serum IgM level using single dilution indirect-ELISA.

Reagents
1. Wash Buffer (PBS with 0.05% tween-20)

2. Coating buffer (0.05M sodium Carbonate buffer, pH9.5)
   \[ \text{Na}_2\text{CO}_3 : 0.159\% \]
   \[ \text{NaHCO}_3 : 0.293\% \]


4. Antibody dilution buffer (pH 7.0): 250 mg of BSA was dissolved in 100 ml of PBS and stored at 4°C.

5. Substrate buffer, 100ml
   0.1 M Citric Acid solution: 24.3ml
   0.2 M Dibasic Sodium phosphate solution: 25.7ml
   Triple Glass Distilled Water: 50ml
   Orthophenyl Diamine: 40mg
   \[ \text{H}_2\text{O}_2 : 40\mu l \]
   The solution is prepared fresh before use.

6. Stop Solution: 1.5N H$_2$SO$_4$

Equipment
Microplate reader
Procedure

The OMV was diluted in coating buffer up to the final concentration of 10 µg per 100 µl (0.1 mg per ml). The plate was sealed with parafilm and incubated at 4 °C for overnight followed by three times washing of the plates with washing solution. The 100 µl of blocking solution was added in each well. The plate was then incubated at room temperature for 1 hour followed by three times washing of the plates with washing solution and then tapping against blotting paper. The 100 µl of 1:100 diluted serum (in antibody dilution buffer) was added in each well. The plate was incubated for 2 hours at room temperature followed by three times washing of the plates with washing solution and then tapping against blotting paper. The 100 µl of anti-mouse HRP conjugated antibody (diluted 1:3000 times in antibody dilution buffer) was added in each well. The plate was then incubated for 1 hour at room temperature. The OPD substrate solution was prepared during the time of incubation (24.3 ml of 0.1 M citric acid, 25.7 ml of 0.2 M dibasic sodium phosphate solution, 40 mg orthophenyl diamine, 0.04 ml of hydrogen peroxide and 50 ml of triple glass distilled water). The plate was washed thrice with washing solution followed by tapping against blotting paper. The 100 µl of OPD substrate solution was added in each well followed by incubation of the plate at room temperature in dark for 30 minutes. The 100 µl of 1.5 N NaOH or 1 N H₂SO₄ was added in each well. The OD was read at 492 nm in the ELISA plate reader.

Troubleshooting

Time Taken

The whole procedure takes about 6 hours.

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