

MEDIUM BINDING CAPACITY SURFACE FOR IMMUNOLOGICAL ASSAYS

A hydrophobic surface suitable for passive adsorption of proteins with different grades of hydrophobicity.

FEATURES

Assays in which the adsorbed molecule present large hydrophobic regions, such as antibodies.

Furthermore this surface is highly selective and shows high affinity towards hydrophobic polypeptides that present a molecular weight > 10kDa.

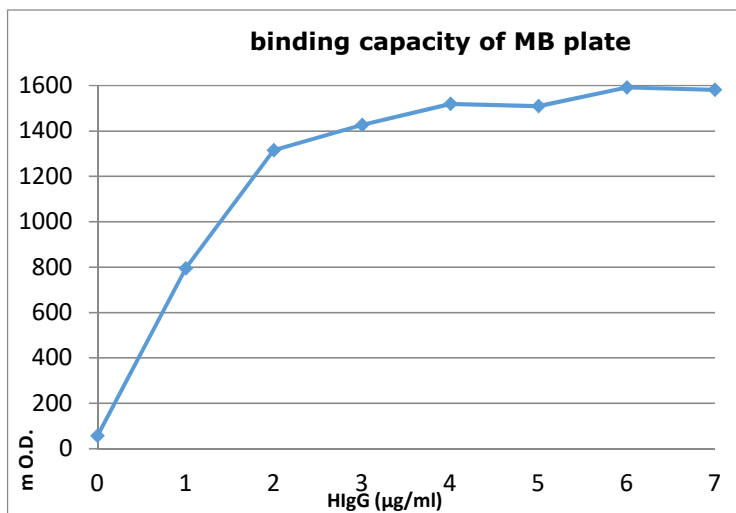
PERFORMANCES

A coating of antibodies of IgG class was carried out to evaluate its usefulness along with to evaluate the binding capacity of this surface.

Method 15

Method 15 is an indirect method with human IgG coated on medium binding plates and then revealed through an AHlgG/POD conjugate.

1. dispense 100 μ l/well of different concentrations of human IgG diluted in 0.1M PBS pH 7.2 : 1-2-3-4-5 μ g/ml and incubate overnight at 4°C
2. wash 3 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
3. dispense 150 μ l/well of BSA 1% in 0.1M PBS pH 7.2 and incubate 2 hours at R.T. for blocking the remaining active sites
4. wash 3 times with 0.1M PBS pH 7.2+ 0.05% Tween[®] 20
5. dispense 100 μ l/well of Goat Anti-HlgG-HRP conjugate and incubate 30' at R.T.
6. wash 3 times with 0.1M PBS pH 7.2 + 0.05% Tween[®] 20
7. dispense 100 μ l/well of TMB
8. after 30' stop the reaction with H₂SO₄ 1 N
9. reading is made at 450 nm



The data show that a plateau has got starting with an IgG concentration of 2.0 μ g/ml.

This concentration means the well binding capacity we can express as:

- μ g/well = 0.200 (200 ng/well/100 μ l)

As 100 μ l of liquid, in term of area , represent 1 cm² it is possible to state that the binding capacity is close to 200 ng/cm². These data are well correlated with other experiments carried out with an unmodified polystyrene surface.