

NO BINDING CAPACITY SURFACES

TECHNICAL NOTES N. 11

A surface that prevents the binding of proteins to the wells. Addressed to those procedures that need to avoid any modification of the activity of the molecules (e.g. enzymes) induced by the reactions that could occur with the well surface.

Two types of tests were made for checking the properties of our **No Binding** capacity surface :

a. No binding capacity against IgG (met. 14)

1. coating: dispense 100µl/well of Rabbit Anti-HiGM (concentration of 25 µg/ml in Carbonate Buffer pH 9.6) and incubate 24 h at 4°C.
2. 4 washing with PBS pH 7.2
3. blocking: dispense 150 µl/well of BSA 1% in PBS pH 7.2 and incubate overnight at 4°C.
4. wash 4 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
5. dispense 100 µl/well of Biotinylated Goat Anti- Rabbit IgG (concentration from 0.062 to 0.0007 µg/ml) and incubate 1 hr at room temperature
6. wash 4 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
7. dispense 100 µl/well of Streptavidin-POD (concentration 0.16 µg/ml) and incubate 1 hr at room temperature
8. wash 4 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
9. dispense 100 µl/well of TMB
10. after 30' stop the reaction with H₂SO₄ 1 N (100 µl/well)
11. reading is made at 450 nm after 5'

results

O.D. values	NO binding	Medium binding	High binding HB 8
<i>RT</i>	62	974	1563
<i>45° C</i>	60	n.a.	1476
<i>- 20°C</i>	61	n.a.	1590

The test was performed after 7 days in the above conditions

b. No binding capacity against Albumin (met. 25)

1. coating: dispense 100µl/well of a mixture of 20 ng/ml Biotinylated Albumin diluted in 3 µg/ml of BSA in 0.1 M PBS pH 7.2 and incubate 24 h at 4°C.
2. wash 4 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
3. dispense 100 µl/well of 0.1 µg/ml Streptavidin-POD and incubate 30' at room temperature
4. wash 4 times with 0.1M PBS pH 7.2 + 0.05%Tween[®] 20
5. dispense 100 µl/well of TMB
6. after 10' stop the reaction with H₂SO₄ 1 N (100 µl/well)
7. reading is made at 450 nm

results

	NO binding	Medium binding
O.D. values	76	392