POLY- L-LYSINE COATED SURFACE

TECHNICAL NOTE N. 22

Electrostatic functional activity

General procedure for binding dsDNA to Poly-L-Lysine coated surface

1. dilute the dsDNA molecule to 1-10 μg/ml in 20 mM TRIS-HCl pH 8, 0.1 mM EDTA
2. proceed with incubation: conditions depend on dsDNA molecular weight and purity
3. wash three times to remove the unbound material
4. proceed with your specific test/application

example of test: human (autoantibodies) IgG determination to dsDNA

1. dilute dsDNA from *Calf tymus* (Sigma code D4522) to 5 μg/ml in 20 mM TRIS-HCl pH 8, 0.1 mM EDTA
2. add 100 μl/well of the diluted dsDNA to each wells and incubate o/n at + 4 °C
3. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
4. add 200 μl to each wells of 0.1 M PBS pH 7.2, 0.5 % BSA and incubate 2 h at room temperature
5. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
6. add 100 μl of diluted human serum with the following IgG concentrations to dsDNA:
7. 0-10-50-150-300 IU/ml
8. incubate 30' at room temperature
9. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
10. add 100 μl of diluted goat anti-human IgG-peroxidase labeled
11. incubate 30' at room temperature
12. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
13. add 100 μl/well of TMB substrate and incubate 15 minutes at room temperature
14. stop the substrate reaction by adding 100 μl of sulphuric acid 1N and read the optical density
15. values at 450 nm