POLY- L-LYSINE COATED SURFACE

Biomat has developed a polystyrene surface with physically adsorbed poly-L-Lysine. The monomeric L-Lysine chain shows a high density of groups:

- α-amino
- α-carboxyl
- ε-amino

These groups are able to react trough electrostatic and stereospecific bonds. The polystyrene optical features don't change, allowing the modified surface to be used as a valid tool to carry out biological tests.
This surface shows its usefulness for these applications:

- **interactions with plasminogen and plasminogen activator**
- **interactions with ribosomal RNA**
- **interactions with double stranded DNA**

TECHNICAL NOTE N. 21

Stereospecific binding activity

General procedure for binding NHS-b to Poly-L-Lysine coated surface

This test is suitable for measuring the available ε- amino groups on Lysine

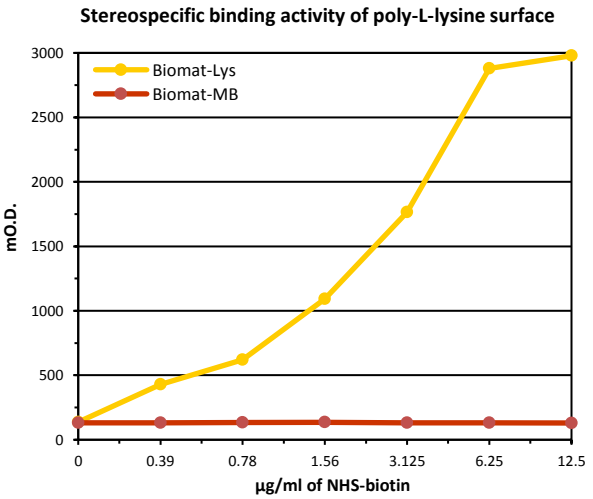
Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MT12F-LYS-L (poly-L-Lysine coated plate) MT0F-MB (medium binding capacity)
ε-Caproylamido-biotin-N-hydroxysuccinimide ester (NHS- biotin)	BIO-SPA	Cat No. B002-61
Dimetilformamide (DMFO)	Fluka	Cat No. 40250
Tween® 20	Merck	Cat No. 822184
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Experiment

1. Dispense 100µl NHS-biotin solutions 12.5 – 6.25 – 3.125 – 1.56 – 0.78 - 0 µg/ml diluted in 0.1M PBS+ Tween® 20 0.15% pH 7.2 into the wells. Seal the wells with adhesive tape to prevent evaporation.
2. Incubate overnight at 4°C.
3. Empty the wells and wash with 0.1M PBS+ Tween® 20 0.05%, pH 7.2 four times.
4. Add 100µl of 50 ng/ml streptavidin-HRP to each well and incubate 30 minutes at room temperature.
5. Empty the wells and wash with 0.1M PBS+ Tween® 20 0.05%, pH 7.2 four times.
6. Add 100 µl /well of TMB substrate solution and incubate 10 minutes at room temperature.
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm.



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