POLY- L-LYSINE COATED SURFACE

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.