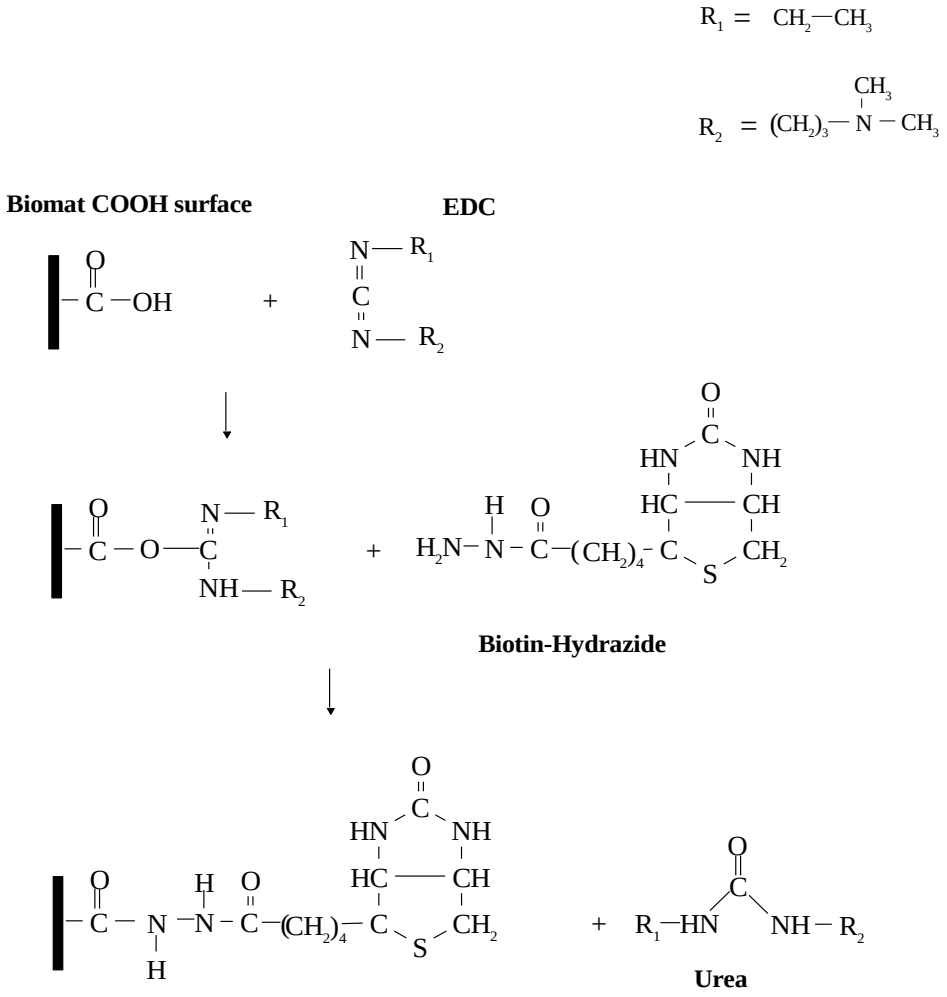
CARBOXYLATED SURFACE

Coupling molecules, having an amino group, to Biomat COOH surface

The amino group presents in any molecules, such as peptides or proteins, binds to Biomat COOH through formation of amide bonds between the amino group presents in the molecule and the surface carboxylic group by the action of carbodiimide.

The figure 1 shows the reaction scheme for coupling of the hapten, biotin-hydrazide, through its available amino group.

Figure 1



Biotin-Hydrazide coupled to Biomat COOH surface

Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MG04F-COOH MG01F-HB (high binding capacity)
Biotin-Hydrazide	Sigma	Cat. No. B 7639
1-Ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC)	Sigma	Cat. No. E 1769
2-Morpholinoethanesulfonic acid (MES)	Fluka	Cat. No. 69889
Dimethylsulfoxide (DMSO)	Merck	Cat. No. 2931
Tween® 20	Merck	Cat. No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Biotin-hydrazide stock solution

Biotin-hydrazide	5 mg
DMSO	to 5 ml

Biotin-hydrazide solution 100 μg/ml

Biotin-hydrazide stock solution	1000 μl
EDC	10 mg
MES 0.1M pH 6.0	to 10 ml

Biotin-hydrazide solution 50 μg/ml

Biotin-hydrazide stock solution	500 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 10 μg/ml

Biotin-hydrazide stock solution	100 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 1.0 μg/ml

Biotin-hydrazide stock solution	10 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.5 μg/ml

Biotin-hydrazide stock solution	5 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.25 μg/ml

Biotin-hydrazide stock solution	2.5 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.1 μg/ml

Biotin-hydrazide stock solution	1.0 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Streptavidin-mix

Streptavidin	50 μg
Streptavidin-peroxidase	0.5 μg
PBS-BSA 1%	10 ml

Experiment

1. Add 100 µl of biotin-hydrazide solutions 100-50-10-1-0.5-0.25-0.1 µg/ml and 100 µl 0.1 MES pH 6.0 as 0 µg/ml to the wells (carboxylated and HB not activated). Seal the wells with adhesive tape to prevent evaporation.
2. Incubate overnight at room temperature
3. Empty the wells and wash with 0.1 M PBS+0.05% Tween® 20 pH 7.2 four times
4. Add 100 µl of streptavidin- mix to each well and incubate 30 minutes at room temperature
5. Empty the wells and wash with 0.1 M PBS+0.05% Tween® 20 pH 7.2 four times
6. Add 100 µl of TMB substrate solution to each well 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

Results

The results of this experiment (fig. 2) clearly show that the molecule (biotin-hydrazide) is bound in a detectable way to the Biomat COOH whereas no detection could be obtained on Biomat HB.
The results indicate that a covalent coupling has taken place between the amino group in the biotin-hydrazide and the carboxylic group grafted on the Biomat COOH.
The results (data not displayed) show that without adding carbodiimide the covalent binding of biotin-hydrazide does not occur.

Figure 2