MALEIMIDE COATED SURFACE

The Biomat product is a 96 well coated microplate with maleimide and treated to block non-specific binding sites and to maintain stable activity.
Maleimide coated surfaces offer a powerful instrument for binding biomolecules containing free sulfhydryl groups (e.g. peptides that contain a terminal cysteine or thiol containing haptens), or reducible disulfide bonds that are difficult to coat onto polystyrene plates. These coated plates are a very useful tool for assays requiring site-directed orientation of particular biomolecules especially during antibody production.
At pH 6.5-7.5 maleimide reacts with free sulfhydryl groups to yeld stable bonds, while the reaction with amine becomes significant at pH > 7.5.
If sulfhydryl-containing peptides and proteins oxidize in solution and form disulfide bonds, they must be preventively reduced to free sulfhydryls for allowing interaction with maleimide.

Product specifications

Available configurations
Individually pouched 96-well microplates, configured in 12 removable 8-well strips.

Coating
A derived maleimide is coated using 100 µl/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

Binding capacity
Microplate was saturated with Glutathione Ethyl Ester Biotin Amide (BioGEE) at a concentration of 2.5 µg/ml (250 ng/well) in an ELISA format using Streptavidin-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details)

The Biomat Maleimide microplate shows a nominal binding capacity of **~ 440 pMol BioGEE/well**.

Uniformity
Microplates show a CV% less than 5 when used as a catcher of Glutathione Ethyl Ester Biotin Amide (BioGEE) in an ELISA format using streptavidin-HRP as detector and TMB as substrate.

Storage and Stability
The microplates, if unopened, are stable refrigerated until the expiration date printed on the label. If opened, store in closed pouch with desiccant and use within the expiration date.

TECHNICAL NOTE N. 35

Binding capacity test

1. Add 100 µl of different concentrations of Glutathione Ethyl Ester Biotin Amide (BioGEE) from 0.1 to 10 µg/ml diluted in 10 mM PBS pH 6.6, 115 mM NaCl, 100 mM EDTA, 40 mM sucrose and incubate for 2 hours at room temperature
2. Empty the wells and wash with ELISA wash buffer (Biomat code 200-3) four times
3. Add 100 µl/well of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml), diluted 1:5,000 and incubate for 45 minutes at room temperature
4. Empty the wells and wash with ELISA wash buffer (Biomat code 200-3) four times
5. Add 100 µl/well of TMB substrate solution (Biomat code 500-1) and incubate 10-15 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl/well of Stop solution (Biomat code 600-1) and read the optical density values at 450 nm

The data show that a plateau has got starting with a BioGEE concentration of 2.5 µg/ml.
This concentration means the well binding capacity we can express as:

- µg/well = 0.25 µg (250 ng/well)
- pMol/well= 440 (this result is calculated considering the BioGEE M.W. = 561 Da)

Figure 1