

BIOTIN COATED SURFACE

Biotin, or vitamin H (MW 244 Da), is a small naturally occurring cofactor that is present in every living cell in very minute amounts (usually less than 0.0001 %). The biotin molecule normally exists bound to proteins (such as pyruvate carboxylase) through its valeric acid carboxylic group by an amide bond to lysine side-chain amines.

Biotin coated surfaces offer a powerful instrument to carry out one of the most useful interactions in immunochemistry that involves the specificity binding of biotin to avidin, streptavidin or neutravidin.

This binding shows a great constant affinity (10^{-15} M).

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 avidin**
- **interactions with streptavidin**
- **interaction with neutravidin**

TECHNICAL NOTE N. 20

Evaluation of binding specificity towards Streptavidin-peroxidase conjugate

1. Dilute streptavidin-peroxidase conjugate from 2000 to 125 ng/ml with 0.1 M PBS pH 7.2 containing 0.2 % BSA
2. Add 100µl of each dilution to the wells of Biotin coated plate and incubate 60' R.T.
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 µl /well of TMB substrate solution and incubate 10 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

