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BIOTIN COATED SURFACES

Biotin, or vitamin H (MW 244,31), 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 the avidin or streptavidin. This binding shows a great constant of 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***

TECHNICAL NOTES N. 20 ***Evaluation of binding specificity towards Streptavidin-peroxidase conjugate***

1. Dilute streptavidin-peroxidase conjugate from 1: 500 to 1: 8000 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' RT. Add the same solutions to abumin coated plate as comparison for evaluate the specificity of binding.
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.