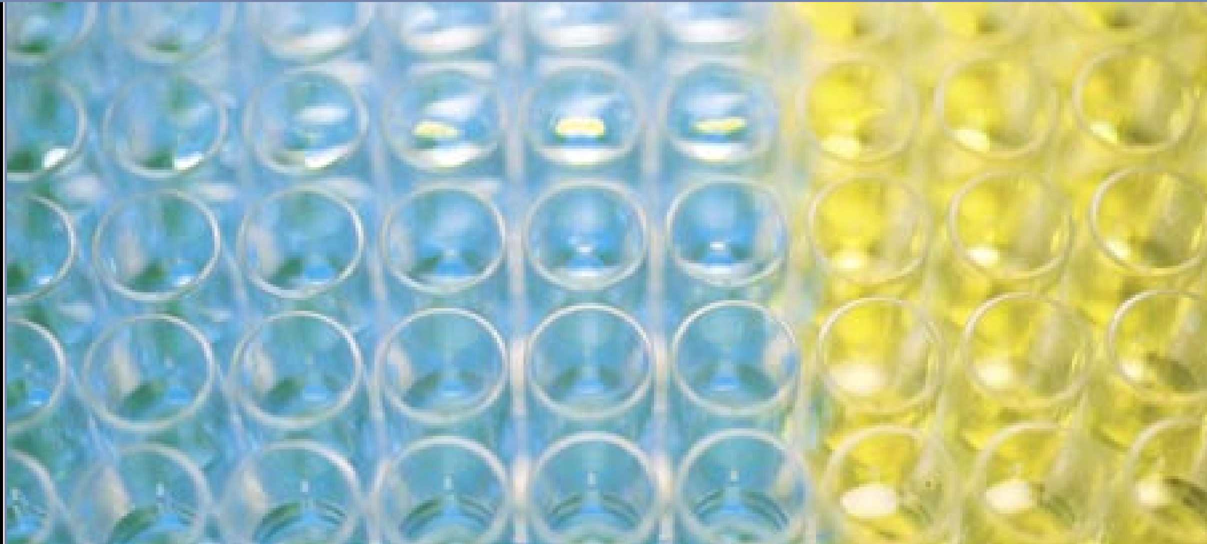
Streptavidin Coated Microtiter® Plates



- Highly specific binding
- Strong, stable interactions

Analyze • Detect • Measure • Control™

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Streptavidin Coated Microtiter® Plates

Characteristics and Structure of Streptavidin

- streptavidin is a tetrameric protein, which can bind four biotins per one molecule; each monomer binds one molecule. (Figure 1)

- biotin binds to streptavidin with a very high affinity ($K_{aff} = 10^{13} M^{-1}$)

- streptavidin/biotin complexes are very stable over a wide range of temperature and pH

- streptavidin doesn't have any electrical charge in neutral or slightly basic pH -> it doesn't cause for example unspecific electrostatic binding

- because the binding of compounds on streptavidin plates is based on the strong affinity of streptavidin towards biotin, the compounds must be biotinylated prior to use

Streptavidin Coated Microplates

Streptavidin forms a good solid phase for binding molecules. This can be utillized by immobilizing streptavidin on the surface of a microtiter plate. When using streptavidin coated microplates for binding molecules for example the orientation of the binding can be controlled, and even small molecules otherwise difficult to bind can be attached on a streptavidin coated surface. After being biotinylated several kinds of molecules can be bound. Applications include:

- proteins
- peptides
- polysaccharides
- DNA/ RNA

The biomolecules may be labelled with different labels and thereafter detected according to the label used either by colorimetric, luminometric or fluorometric methods. (Figure 2.)

Suggestions for General Assay Protocols for Binding Biomolecules onto Streptavidin Coated Microplates

Peptide and Protein Binding

1. Prepare a solution of the biotinylated protein or peptide in bicarbonate, PBS or TBS buffer. Start with concentration of 1 – 10 µg/ml if the optimal concentration is not known.

2. Add 50 – 200 µl of the solution per well and allow the samples to incubate for 15 – 60 minutes at 25 – 37 °C.

3. Wash the wells three times, 300 µl per well, with PBS or TBS + 0.05 % Tween-20.

4. Incubate the wells with 100 – 200 µl of an appropriately diluted primary antibody in PBS or TBS + 0.05 % Tween-20 for 30 – 60 minutes.

5. Wash the wells three times, 300 µl per well, with PBS or TBS + 0.05 % Tween-20.

6. Incubate the wells with 100 – 200 µl of an appropriately diluted enzyme labeled secondary antibody in PBS or TBS + 0.05 % Tween-20 for 30 – 60 minutes.

7. After adding an appropriate substrate solution, 100 – 200 µl per well, the wells are ready for detection by a suitable method.

PCR Products

1. Prepare the biotinylated PCR product for addition onto streptavidin coated microplate by diluting the reaction 1:10 – 1:50 in PBS + 0.05 % Tween-20.

2. Apply 50 – 200 µl per well of the diluted product and allow the sample to incubate for 15 – 60 min-

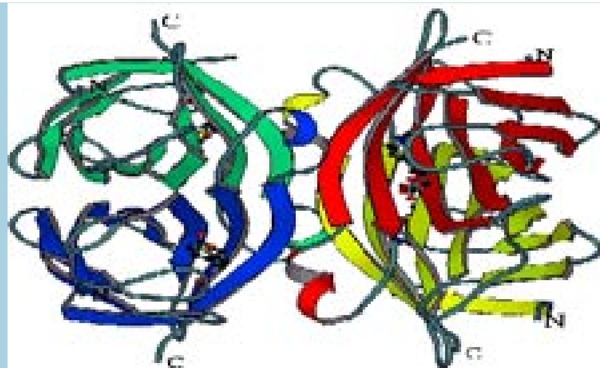

Figure 1:Streptavidin is a tetrameric protein (4 x 13 kDa). Each monomer of streptavidin binds one molecule of biotin.

Enzyme label	Substrate		
	Photometric	Fluorometric	Luminometric
Peroxidase	TMB, ABTS	HPPA	Luminol/ Iodophenol/ Peroxide
Alkaline Phosphatase	pNPP	MUP	Dioxetanes
ß-Galactosidase			Dioxetanes

Figure 2: TMB = 3,3´,5,5´-Tetramethylbenzidine , ABTS = 2,2´-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) , HPPA = 3-p-Hydroxyphenylpropionic Acid , pNPP = p-Nitrophenyl phosphate MUP = 4-Methylumbelliferyl phosphate

utes at 25 – 37 °C.

3. Denature the bound PCR products by adding 100 µl per well of 0.5 M NaOH. Incubate for 5 – 10 minutes.

4. Wash the wells three times, 300 µl per well, with PBS + 0.05 % Tween-20 to remove the nonbiotinylated, complimentary strand of the PCR product.

5. Add 100 – 200 µl per well of a labeled oligonucleotide which is complimentary to the biotinylated strand. Use 0.05 – 0.5 pmole of labeled oligonucleotide per well. Hybridize in the presence of 5X SSC, 0.3% Tween-20, 1 % BSA. Allow the hybridization to proceed for 30 – 60 minutes at 37 – 50 °C.

6. Wash the wells three times, 300 µl per well, with PBS + 0.05 % Tween-20.

7. Add 100 µl per well of an appropriately diluted detection conjugate in PBS + 0.05 % Tween-20. Incubate for 30 – 60 minutes at RT.

8. Wash the wells five to six times, 300 µl per well, with PBS + 0.05 % Tween20.

9. Proceed to the use of the specific substrate for detection.

Optimizing the Protocols

The most important areas to concentrate on when optimizing the procedures are:

1. Nonspecific binding

Means of trying to reduce non-specific binding:

- changing the conjugate concentration

- modifying the washing buffers with components within the ranges suggested below (Figure 3)

2. Washing conditions

To reduce non-specific reversible binding at least three washing steps are recommended.

3. Antibodies and conjugates

Commercially obtained conjugates should be used at the concentrations recommended by the supplier or alternatively they can be optimized for a particular assay.

Troubleshooting

1. Weak signal or no signal at all

- check instrument settings

- check activity of marker enzyme/ molecule

- check conjugate buffer for incompatible components

- check protocol (incubation times, temperatures, buffers etc.)

- check concentrations of primary antibody/ antigen

- check substrate for correct storage conditions or possible contamination

2. High background signal

- check number of washes, interval between washes

- modulate concentrations for primary/secondary antibody

- try chancing the additives in the buffers to block non-specific binding

Detergents	0.05-0.1% Tween-20, 0.002-0.05% Tween 65
Salts	0.5-1.0 M NaCl or Na2HPO4
Protein blockers	0.1-1% BSA or casein
Non protein blockers	1% PEG 20 or Polyvinylpyrrolidone

Figure 3: Washing buffer components

Vocabulary

Avidin
- a tetrameric glycoprotein present in egg white
- positively charged and 66 000 daltons in size.
- isoelectric point about 10.5

Streptavidin
- a tetrameric protein present in bacteria
- isoelectric point near neutral
- size is 60 000 daltons

Biotin
- a vitamin soluble in water
- binds to avidin and streptavidin very specifically and with high affinity, $K_{aff}=10^{13}$ M^{-1}

DNA
- Deoxy-ribonucleic acid
- a double stranded helix that is made up of four nucleotides:
- guanine (G) , cytosine (C) , adenine (A) , thymine (T).
- G and C match together and so do A and T
Hybridization
- two pieces of single stranded DNA (ssDNA) matching perfectly to one another meet and bind together = hybridization
- specificity increases with the length of the DNA strand.

Oligonucleotide
- small piece or sequence of single stranded DNA commonly 15 - 20 nucleotides long (15 mer - 20 mer), but can be up to 100 nucleotides long
- oligonucleotides are used as “primers” in PCR reactions or as “DNA-probes”

DNA-probe
- an oligonucleotide labeled with a suitable label (biotin, enzyme-conjugates, fluorochromes etc.)
- used for finding it’s corresponding strand that is the “target sequence”

Primer
- used in an oligonucleotide PCR reactions as a starting point for DNA elongating
- the area of DNA that needs to be amplified is marked by a primer, which is designed to attach to an opposite strand

Cat.no	Product description	Stripformat
95 029 263	BioBind Strip Assembled	Solid 1x8
95 029 293	BioBind Strip Assembled	Breakable 1x8
95 029 283	Black BioBind Strip Assembled	Solid 1x8
95 029 273	White BioBind Strip Assembled	Solid 1x8
95 029 303	White BioBind Strip Assembled	Breakable 1x8
Each plate in boxes of 5		

Technical characteristics and handling

- The coated area is 200 µl
- Binding capacity determined by FITC-biotin * binding test is according to specifications ≥ 12 pmol of FITC-biotin bound per well
- Storage at +4˚C - +8˚C
- Shelf lives: clear plates 24, black plates 29 and white plates 26 months
- The plates are ready to use

*FITC = Fluorescein Isothiocyanate

References:

1. Labsystems Research Group (1993).Streptavidin Coated Microplates – Use in Solid Phase Immunoassays, Use in Molecular Biology.
2. Freitag, S. (1998). Streptavidin Project Description, http://faculty.washington.edu/stenkamp/stefanieweb/abstract.html

Thermo Electron Corporation
Ratastie 2, P.O. Box 100
FIN-01621 Vantaa, Finland
Tel. +358-9-329 100
Fax +358-9-3291 0414
www.thermo.com

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