Application Note

Streptavidin Coated Microtiter microplates for PCR-ELISA

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This technical note provides a general protocol for performing PCR-ELISA analysis using streptavidin coated Microtiter microplates.

Introduction

Streptavidin is a tetrameric protein, which can bind four biotins per one molecule; each monomer binds one molecule (Fig. 1). Biotin binds to streptavidin with a very high affinity (Kaff ~ 1013 M-1).

Streptavidin coated Microtiter microplates can be used for binding of biotinylated molecules, including

- proteins
- peptides
- polysaccharides
- DNA / RNA

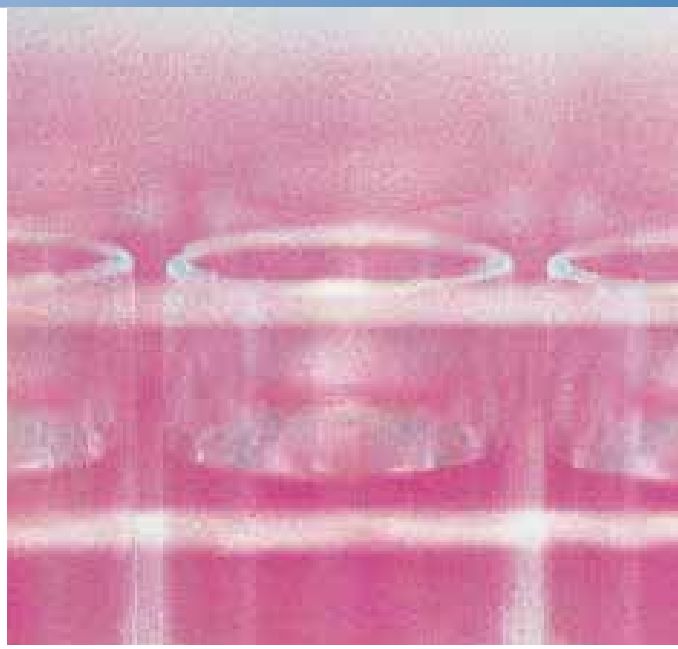
The molecules may be labeled with different labels and thereafter detected according to the label used either by colorimetric, luminometric or fluorometric methods. Thermo Electron offers clear, white and black streptavidin coated Microtiter microplates for these different detection methods.

In this technical note a general protocol for PCR-ELISA is given.

What is PCR-ELISA?

PCR-ELISA is a capture assay for nucleic acids that mimics enzyme linked immunosorbent assay (ELISA). It uses an oligonucleotide capture probe that is labeled with biotin and bound to a streptavidin coated microtiter microplate. The PCR product labeled e.g. with digoxigenin is added and the hybridized product (probe-PCR product) is detected and quantitated with an enzyme-conjugated anti-label antibody (e.g. anti-digoxigenin peroxidase).

PCR-ELISA's have been in use since the late 1980s and have developed into an assay for detecting specific sequences within PCR products. Though many methods are available for detecting specific sequences, PCR-ELISA is useful for detecting and differentiating between multiple targets (2).



The assay is quick and sensitive and allows simultaneous testing of a large number of samples.

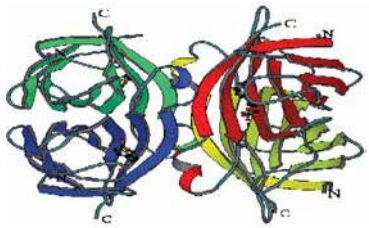


Figure 1 Structure of streptavidin (1).

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General Protocol for PCR-ELISA

PCR-ELISA consists of the following steps:

1. Label the PCR products, e.g. with digoxigenin using a commercial kit. Denature the labeled PCR products, e.g. by adding 100 µl per well of 0.5 M NaOH. Incubate for 5 – 10 minutes.
2. Prepare the biotinylated oligonucleotide probe for addition onto streptavidin coated microtiter microplate by diluting the reaction 1:10 – 1:50 in PBS + 0.05 % Tween-20.
3. Apply 50 – 200 µl per well of the diluted probe and allow the sample to incubate for 15 – 60 minutes at 25 – 37 °C.
4. Wash the wells three to six times, 300 µl per well, with PBS + 0.05 % Tween-20.
5. Add 100 – 200 µl per well of denaturated PCR products, 0.05 – 0.5 pmol per well. Hybridize in the presence of 3-5X SSC, 0.3 % Tween-20, 1 % BSA. Allow the hybridization to proceed for 30 minutes up to 2 hours at 37 – 55 °C.
6. Wash the wells three to nine times, 300 µl per well, with PBS + 0.05 % Tween-20.
7. Add 100 – 150 µl per well of an appropriately diluted detection conjugate (e.g. antidigoxigenin-Fab-peroxidase) 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 % Tween-20.
9. Add a specific substrate for the conjugate and perform the measurement.
10. Examples of PCR-ELISA protocols can be found from references 3, 4 and 5.

Optimizing protocols

Important areas to concentrate on when optimizing the procedures are:

1. Non-specific binding. Means of trying to reduce non-specific binding:
   - changing the conjugate concentra tion
   - modifying the washing buffers with components within the ranges suggested below (Table 1)
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 opti¬mized for a particular assay.

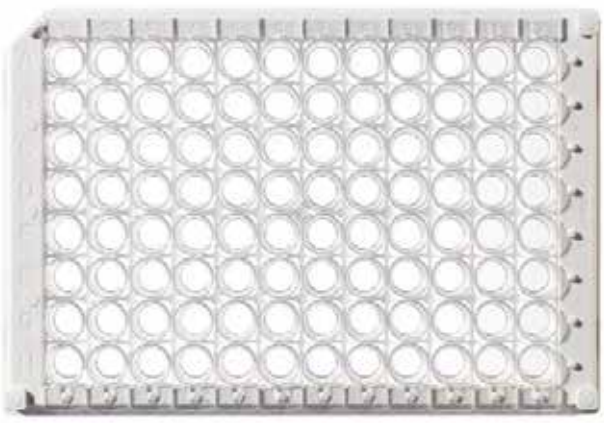
Detergents	0.05-0.1 % Tween-20, 0.002-0.05 % Tween-65
Salts	0.5-1.0 M NaCl or Na2HPO4
Protein blockers	0.1-1 % BSA or casein
Non-protein blockers	1 % PEG 20 or Polyvinylpyrrolidone

Table 1 Washing buffer components

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 microplates 24, black microplates 29 and white microplates 26 months
- The microplates are ready to use

* FITC = Fluorescein Isothiocyanate



References

1. http://faculty.washington.edu/stenkamp/stefanieweb/abstract.html
2. http://www.btc-bti.com/pcrelisa.htm
3. Garcia L, M Alonso-Sanz, M J Rebollo, J C Tercero and F Chaves. 2001. Mutations in the rpoB gene of Rifampin-resistant Mycobacterium tuberculosis isolates in Spain and theri detection by PCR-enzyme-linked immunosorbent assay. J. Clin. Microbiol. 39, 1813-1818.
4. Laitinen R, E Malinen and A Palva. 2002. PCR-ELISA I: Application to simultaneous analysis of mixed bacterial samples composed of intestinal species. System. Appl. Microbiol. 25, 241-248.
5. Malinen E, J Mättö, M Salmitie, M Alander, M Saarela and A Palva. 2002. PCR-ELISA II: Analysis of Bifidobacterium populations in human faecal samples from a consumptional trial with Bifidobacterium lactis Bb-12 and a galacto-oligosaccharide preparation. System. Appl. Microbiol. 25, 249-258.

Cat. no	Product description	Bottom	Strip	Qty/bag	Qty/box
95 029 263	BioBind Strip Assembled	Flat	Solid 1x8	1	5
95 029 293	BioBind Strip Assembled	Flat	Breakable 1x8	1	5
95 029 273	White BioBind Strip Assembled	Flat	Solid 1x8	1	5
95 029 283	Black BioBind Strip Assembled	Flat	Solid 1x8	1	5

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