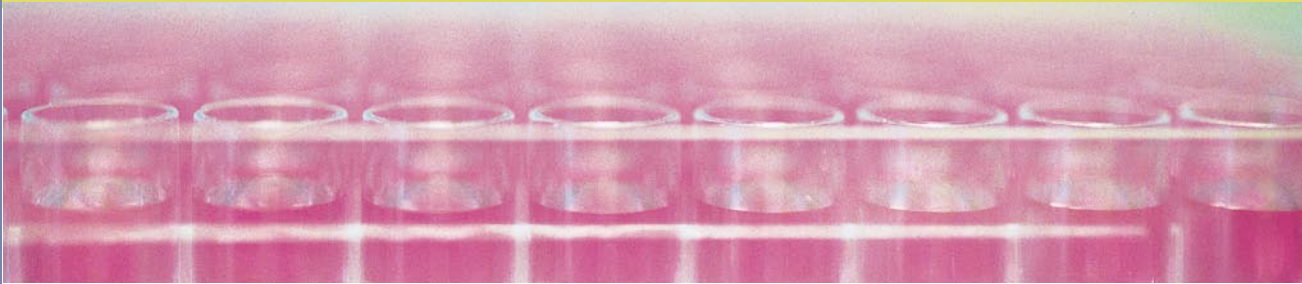


Streptavidin Coated Microtiter microplates for PCR-ELISA



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

LCP Research Group, Thermo Electron Oy, Vantaa, Finland

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 ($K_{\text{aff}} \sim 10^{13} \text{ M}^{-1}$).

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, luminescent 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.

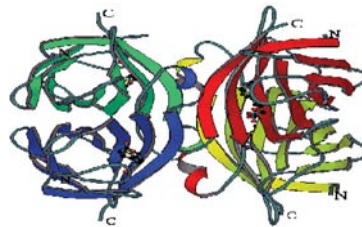


Figure 1 Structure of streptavidin (1).

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.

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 $^{\circ}\text{C}$.
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 denatured 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.

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 concentration

- 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

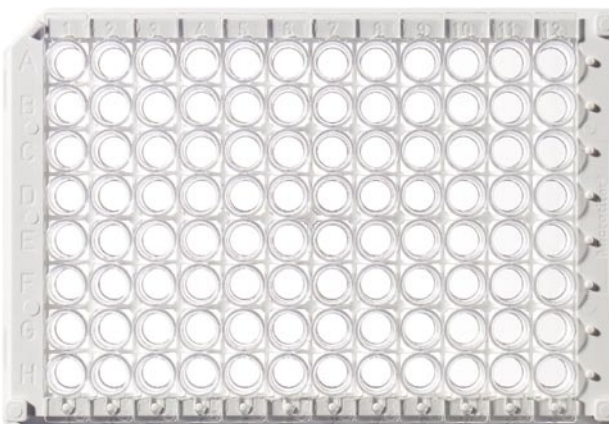
Commercially obtained conjugates should be used at the concentrations recommended by the supplier or alternatively they can be optimized for a particular assay.

Table 1 Washing buffer components

Detergents	0.05-0.1 % Tween-20, 0.002-0.05 % Tween-65
Salts	0.5-1.0 M NaCl or Na ₂ HPO ₄
Protein blockers	0.1-1 % BSA or casein
Non-protein blockers	1 % PEG 20 or Polyvinylpyrrolidone

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 their 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 Salmite, 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

© 2006 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

North America: USA/Canada +1 866 984 3766

Europe: Austria +43 1 801 40 0, Belgium +32 2 482 30 30, Finland +358 9 329 100, France +33 2 2803 2000, Germany national toll free 08001-536 376, Germany international +49 6184 90 6940, Italy +39 02 95059 1, Netherlands +31 76 571 4440, Russia/CIS +7 095 225 11 15, Spain/Portugal +34 93 223 3154, Switzerland +41 44 454 12 12, UK/Ireland +44 870 609 9203

Asia: China +86 21 6865 4588 or +86 10 5850 3588, India +91 22 5542 9494, Japan +81 45 453 9220, Other Asian countries +852 2885 4613

Countries not listed: +49 6184 90 6940 or +33 2 2803 2000

www.thermo.com