Activated Slides

for DNA Microarrays

Technical product information

Immobilization of DNA

Oligonucleotide and cDNA microarrays have become widely used tools for the investigation and functional interpretation of sequence information. With most of these applications optimizing the surface chemistry of the substrate is key in attaining high signal to background (S/B) ratios and specificity.

Most substrate coatings are silane based monolayers, containing either amino functionalities for electrostatic interaction with the negatively charged DNA backbone or aldehyde, epoxy and activated carboxyl groups for covalent attachment of amino modified oligos.

Limitations of current slide substrates

- DNA strands align themselves parallel to the surface as they are electrostatically attracted. This affects the hybridization's specificity and results in significant non-specific sample adsorption. Therefore the majority of DNA microarrays require a blocking step after spotting. Nevertheless, very often the background is still too high.
- The efficiency of immobilization is moderate and varies depending on the size of the probe-oligos.
- DNA density on all of these coated substrates cannot exceed a monolayer that limits the absolute signal intensity.
- State-of-the-art three-dimensional coatings can immobilize higher DNA quantities, but the resulting higher signal comes at the expense of significantly increased background fluorescence, so the S/B ratio isn't improved.

XanTec CX slides eliminate these limitations

To overcome the disadvantages of currently used substrate coatings, XanTec bioanalytics has developed the CX surface - a three-dimensional activated polycarboxylate nanocoating - which allows covalent attachment of aminomodified DNA in a multilayer fashion. Due to the negative charge and extreme hydrophilicity of this hydrogel, the CX surface is inert towards DNA, so the non-specific background is drastically reduced. The covalent immobilization is highly efficient and not dependent on the length of the DNA, thereby enabling stable coupling of short synthetic oligonucleotides to polynucleotides of several hundred bases.

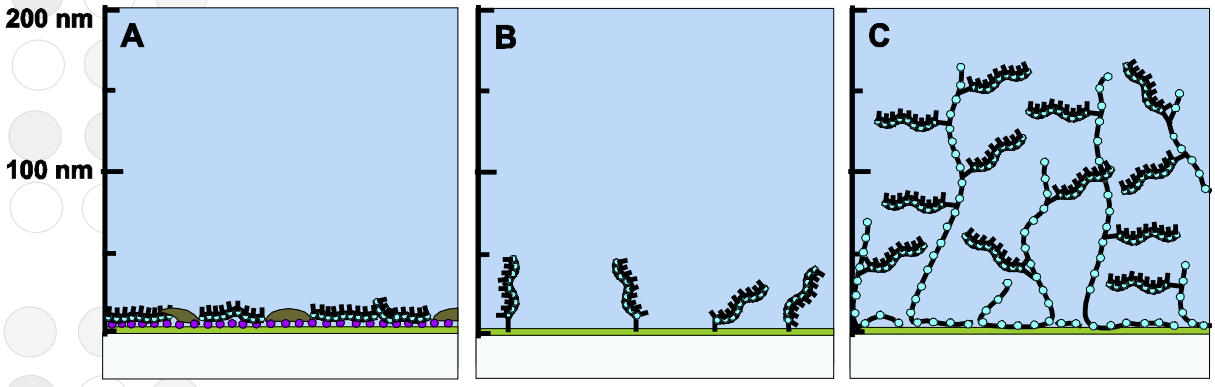

Fig.1: Oligonucleotide orientation after immobilization on different surfaces:

A: Electrostatic immobilization on aminosilane or poly-l-lysine coatings with protein blocking.
B: Covalent attachment to aldehyde, epoxy or activated carboxyl silane monolayers.
C: Covalent multilayer immobilization to XanTec's 3D activated CX hydrogel nanocoating.

The surface is moderately hydrophobic before spotting, thus yielding good spot morphologies and allowing a small pitch, i.e. spot to spot distance. After spotting and hydrolysis of residual reactive groups, the surface becomes very hydrophilic and highly negatively charged.

As a result, the terminally attached and electrostatically repelled probe oligos orient perpendicular to the immobilization matrix. This bottle brush structure enhances the probes' accessibility with respect to the complementary target strand. In combination with the minimal background and a very low substrate autofluorescence, these surface properties yield a superior signal to noise ratio compared to existing state-of-the-art DNA microarray slides. Furthermore, the inert characteristics and spacer functionality of the CX coating opens the possibility of regeneration and reuse of hybridized microarrays.

XanTec CX slides effectively:

- achieve higher signal to background ratios
- increase specifity
- eliminate blocking steps and save time.

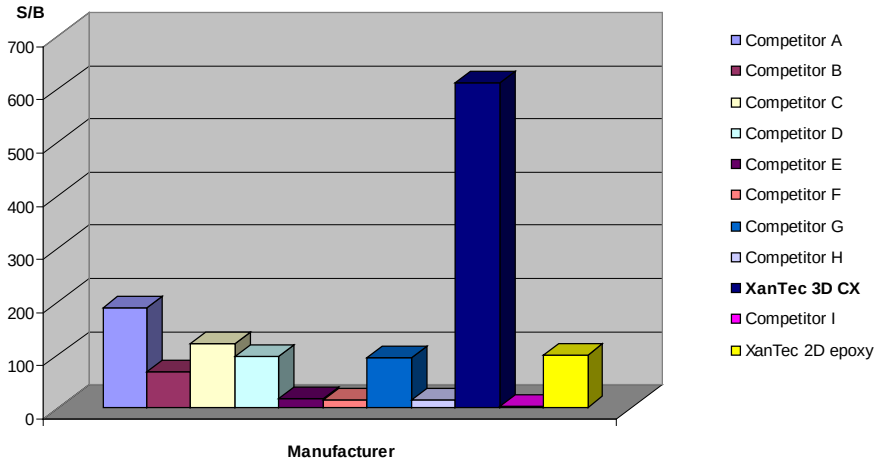

Fig. 2: Frequently used slides for DNA microarray production from different manufacturers were checked for signal and background intensity after spotting and hybridisation of 20-mer DNA oligos following manufacturer's protocols. A side by side comparison shows the improved signal / background ratio of the CX surface.

Technical data CX slides

Glass type	Borosilicate glass with low autofluorescence
Dimensions	76.0 x 25.0 mm ± 0.2 mm
Thickness	1.00 mm ± 10 µm
Flatness	< 2 µm
Parallelism	< 5 arcsec
Surface treatment	Optically polished
Edge specification	Cut, slightly seamed
Coating	Both sides completely coated
Spottable area	60 x 21 mm

XanTec CX slides are available in 5 and 50 unit packs. All slides are optically polished and come ready to spot, together with a detailed protocol.

Protein microarray substrates are described in the separate product information ‘Coated Slides for Protein Microarrays’.

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