

Comparison of SAL and epoxy silane coated slides for microarray production

Asper Biotech produces SAL type (amino silane with linker) of microarray glass slides for binding of aminated oligonucleotides (8-75mer).

SAL slides have 3-Aminopropyl trimethoxysilane plus 1,4-Phenylene di-isothiocyanate coating.

Epoxy slides have 3-Glycidoxypropyl trimethoxysilane coating.

Both surfaces bind oligonucleotides with 5' or 3' amino groups under alkaline conditions.

The following criteria for the two activation methods of glass surface were compared:

- the relative amount of specific and nonspecific binding of oligonucleotide probes
- the efficiency of hybridization of attached oligonucleotide probes
- the efficiency of both slide types for arrayed primer extension
- the extent of nonspecific binding of oligonucleotide probes
- storage stability of activated slides

The **binding efficiency** of probes to activated glass surface was estimated by immobilization experiments using equal (50 μ M) concentrations of a 25mer oligonucleotide with 5'(C12) amino modification.

The results showed that the binding efficiency of probes on SAL slides is as high as ~70%, exceeding the binding efficiency of epoxy slides (~25%) by more than twofold.

The **efficiency of hybridization** on the slides was tested with a 25 mer oligonucleotide probe using a fluorescently labeled oligonucleotide complementary to the immobilized probe as a target. The signals from SAL slides were 10 times higher compared to signals from epoxy slides processed under identical conditions (Fig.1 and 2).

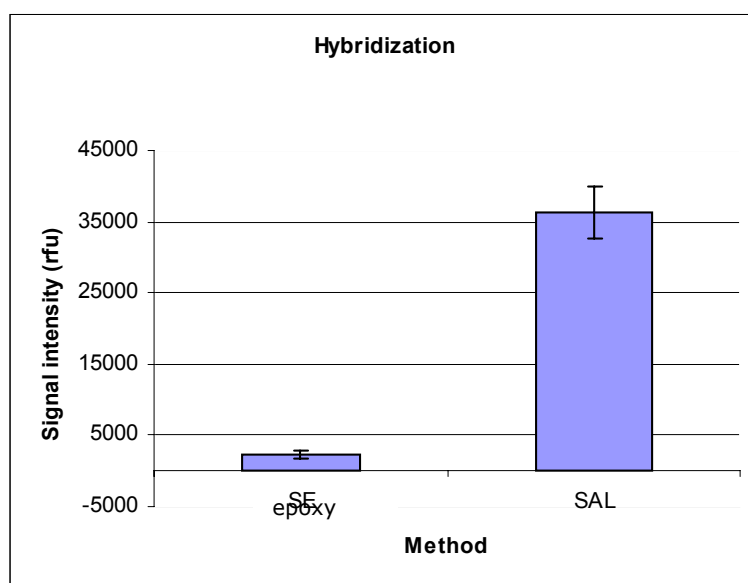


Fig.1. Comparison of hybridization efficiency on epoxy and SAL slides. The results are calculated as mean relative fluorescence (rfu) values obtained from 120 measurements for both slide types. Error bars represent standard deviations

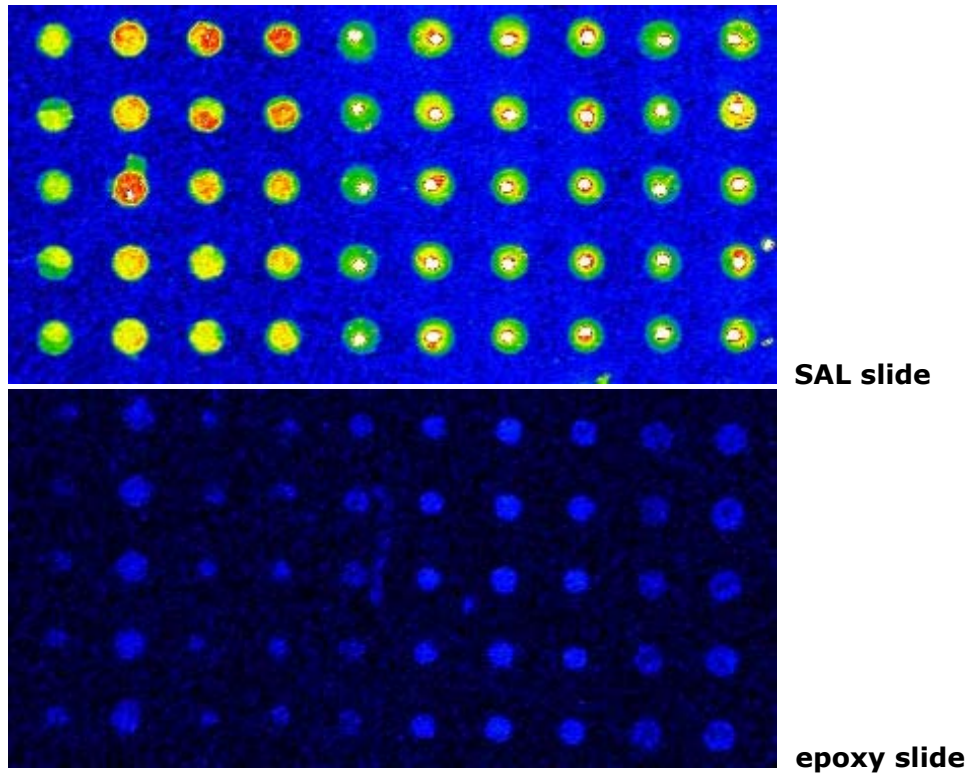


Fig.2. The hybridization efficiency on SAL and epoxy slides processed under identical conditions.

SAL surface was also proven to have advantages in **arrayed primer extension** reactions (Fig.4). The signals detected were at least twice stronger compared to epoxy slides.

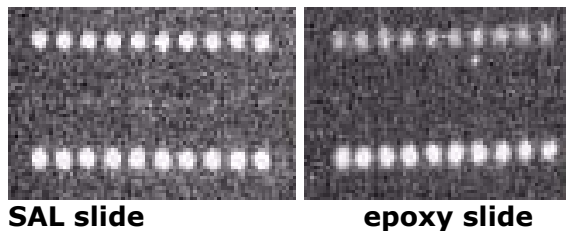


Fig.4. Arrayed primer extension reactions on SAL and epoxy slides.

The extent of **nonspecific binding** was measured via parallel spotting of amino modified and non-modified oligonucleotide probes. Both probes were hybridized with fluorescently labeled target oligo. The level of nonspecific binding was slightly higher on SAL glass surface compared to epoxy surface but was not significant in general (Fig.3).

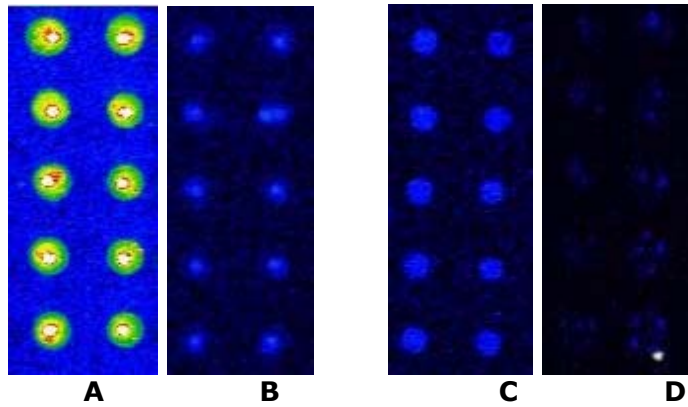


Fig.3. Binding comparison of 5' amino modified vs. nonmodified oligonucleotide probes. Both slides are hybridized with fluorescently labeled target oligo.

- A- binding of 5' amino modified oligonucleotide on SAL slide;
- B- binding of nonmodified oligonucleotide on SAL slide;
- C- binding of 5' amino modified oligonucleotide on epoxy slide,
- D- binding of nonmodified oligonucleotide on epoxy slide.

The **storage stability** of SAL slides (at least 6 months) was lower compared to epoxy type of slides. The spotted epoxy slides were usable even after 10 months of storage. This can be explained with a low reactivity of epoxy groups on the surface.

In **conclusion**, SAL glass slides have better binding efficiency and functional efficiency on both hybridization and arrayed primer extension reactions compared to epoxy slides. However epoxy chemistry is more stable for prolonged storage.

SAL advantages compared to epoxy slide:	Epoxy advantages compared to SAL:
<ol style="list-style-type: none"> 1. SAL slides have at least 2 times better binding capacity of aminated DNA than epoxy coated slides 2. As SAL slides are more reactive, the probe immobilization time to SAL is much shorter; only 2 hours (24 hours to epoxy slides) 3. Lower pH of the printing solution. (epoxy – pH 10.0 required to activate the surface. SAL – pH 9.0) 4. Binding is further from the surface due to the linker molecule, so in theory there is more space for probe binding 	<ol style="list-style-type: none"> 1. Stable coating, long storage time. 2. Blocking of active groups is not necessary after printing.