DNA IMMOBILIZATION ON EPOXY EP SLIDES

Instructions for use

GENERAL INFORMATION

EP slides are coated with dense layer amine reactive epoxy functionalities. Their reactivity is relatively moderate and thus the slides can be exposed to a humid atmosphere over several hours. However, the slide packs should be opened not longer then 2 hr prior to spotting. Opened bags can be stored in a tightly closed receptacle over molecular sieve or other desiccant.

Please note that the slide surface is hydrophilic so depending on the spotter it might be necessary to reduce the spotted volume.

MATERIALS

- EP Slide
- Slide orientation and handling: EP slides are activated on both sides. It is recommended not to spot on surfaces which are facing towards the wall of the transport box. The slides should be handled with tweezers. Avoid touching the surface.
- Spotting solution: 0.1–1 µg amino functionalized DNA/µL spotting buffer. Ensure that the DNA is of high purity (see note below).
- Spotting buffer: The recommended buffer is 10 mM sodium borate, pH 8.5. Other buffers like PBS, SSC or carbonate also give reasonable results but keep in mind that the epoxy reaction occurs at elevated pHs. The use of Tris, or amine-containing buffers must also be avoided since they will compete with your probes for attachment sites.
- Washing buffer A: TBS, 0.05 % Tween-20
- Washing buffer B: 0.5 M KCl, 0.1 M Na-citrate pH 5.0
- Blocking buffer: 1 % BSA in PBS or other commonly used blocking solutions.

Note: Lyophilized oligos are frequently contaminated with ammonia or methylamine traces which affect the coupling reaction. Both issues can be addressed by dissolving the oligo in 200 mM Na_2SO_4 pH 7 and desalting this solution against spotting buffer.

SPOTTING PROTOCOL

1. 30 min before use take the aluminium bag out of the freezer and let it reach room temperature.
2. Remove the slides from the container with tweezers or powder-free gloves. Don't touch or scratch the surface as this will adversely affect the immobilization process.
3. Place the slides in a spotter and spot oligos. Recommended humidity is 10–50 %.
4. Incubate printed microarray slides in humidity chamber (>90 % relative humidity) at room temperature for 30 min.
5. Let the spots dry and incubate for 1 h at 80 °C.

WASHING AND BLOCKING

Washing the slides removes unbound DNA which might interfere with subsequent hybridization experiments. Wash successively 2x 5 min each with washing buffer A and B, rinse quickly with dd water and apply blocking buffer. Incubate at least 30 min on a shaker and finally rinse with dd water. Dry or proceed directly with denaturation. Do not dry the slides between washing steps and between washing and blocking.

DENATURATION STEP FOR ARRAYS SPOTTED WITH PCR-PROBES

This step separates double stranded PCR products into mostly single stranded DNA and allows future hybridization. Although the baking process used to immobilize DNA has already opened the double stranded DNA to some extent, additional denaturing will result in a significant higher hybridization signal.

1. Transfer slides to boiling dd water bath for 2 minutes.
2. Quickly dry the slides using clean dry air or by centrifugation.

Slides are ready for hybridization. The arrays can be stored under nitrogen or argon in a dark cool place over several months. Before hybridisation rehydrate the slides 20 min with hybridisation buffer.

HYBRIDISATION

Several commonly used buffer systems were tested for hybridisation and gave equally good results. Generally 2–5 x SSC works well. Depending on the length of the oligos up to 50 % formamide may be added to increase the specifity.

For more information contact us:

XanTec bioanalytics GmbH

Merowingerplatz 1a
D-40225 Düsseldorf
Germany

Phone +49 211 993 647 44
Fax +49 211 993 647 46
E-mail info@xantec.com

www.xantec.com