Optimising immunohistochemistry and *in situ* hybridsation results by surface acoustic wave microagitation

Based on SlideBooster technology.

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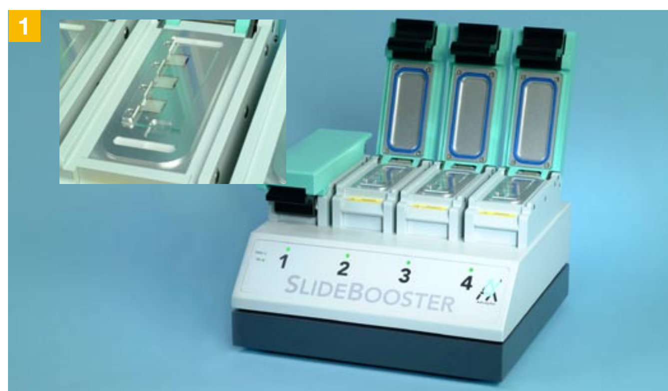
Immunohistochemistry (IHC) and *in situ* hybridisation (ISH) are well established and widely used techniques in laboratory and medical diagnostics. Saving assay time and reducing reagent costs are the key requirements of all new approaches focusing on improvement of these methods. We compared standard manual methods with antibody or probe incubation in hybridisation ovens to the SlideBooster instrument (Advalytix AG, Brunnthal/ Germany) that uses surface acoustic waves for agitating small sample volumes either in a capillary gap or in open droplet geometries. The key feature of this technique is the contamination free mixing with chip-based nanopumps located below the microscope slide. Due to the very low energy input in this system, no destruction of the tissue sections occurs.

Materials & Methods

We used 5 µm consecutive paraffin sections of bovine uterus fixed on Superfrost Plus microscope slides (Menzel, Braunschweig/Germany). The sections were immersed in xylene and then rehydrated through a graded series of alcohol and rinsed in distilled water. Antigen retrieval was carried out by incubation in 10 mM citrate buffer (pH 6.0) at 95°C for 60 min in a waterbath, followed by a 5 min protease treatment.



SlideBooster hybridisation station with acoustic agitation chips below the slide (insert)

For primary detection in IHC we used a rabbit polyclonal estrogen receptor α specific antibody (Santa Cruz Biotechnology, Heidelberg/Germany). Incubation time was 16 hours at room temperature.

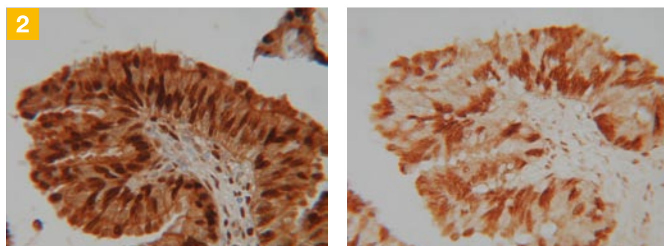
Detection steps were carried out with a biotinylated anti-rabbit secondary antibody in combination with the StreptABComplex/HRP system and DAB+ liquid (Dako Cytomation, Hamburg/Germany).

ISH experiments were performed using a custom-made 20mer biotinylated oligonucleotide probe specific for TRA1 (tumor rejection antigen). Hybridisation time was 16 hours at 38°C in DNA in situ hybridisation solution (Dako Cytomation). Detection steps were carried out with the StreptABComplex/ HRP system in combination with DAB+ liquid according to the manufacturers manual.

Results

Optimising signal intensity in IHC experiments:

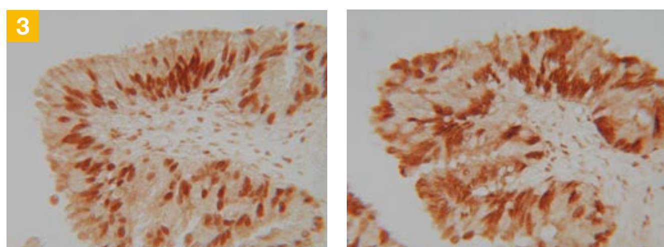
A dilution series for the primary antibody from 1:400 down to 1:10000 showed a possible reduction of antibody concentration by a factor of five between the manual incubation and the agitated experiment in the SlideBooster instrument.



Comparison of a 1:400 dilution with SB400 on left (mix power 27; on:off ratio 7:3) and a manual IHC on the right

The 1:400 dilution in the SlideBooster showed a significant increase in signal intensity compared to the manual experiment (fig. 2).

A dilution of 1:400 with standard incubation resulted in about the same signal as 1:2000 with agitation (fig. 3).



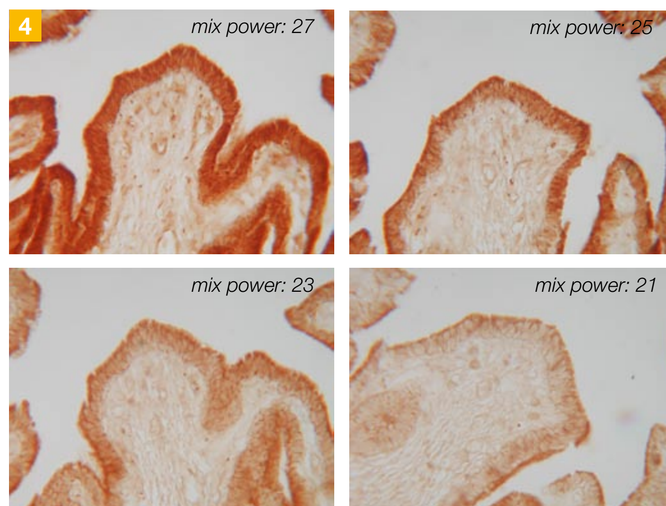
Comparison of a 1:2000 dilution with SB400 on the left (mix power 27; on:off ratio 7:3) and a manual IHC 1:400 dilution on the right

Optimising mixing parameters for ISH in the SlideBooster

The BoosterControl software allows the user to set different mixing power and various on/off times for the mixing in seconds. These possibilities have been integrated to provide the user with the ability to adapt the reaction parameters to the demands of the binding kinetics of the specific biological system.

Determining the optimal mixing power is the key factor for every successful experiment. Applying too low power leads to a loss of potential signal intensities while over-powering may result in signal loss by interfering with binding reactions.

To get optimal results, a mix power of 27 should be selected in combination with an on/off ratio of 7:3 for ISH experiments (fig. 4)

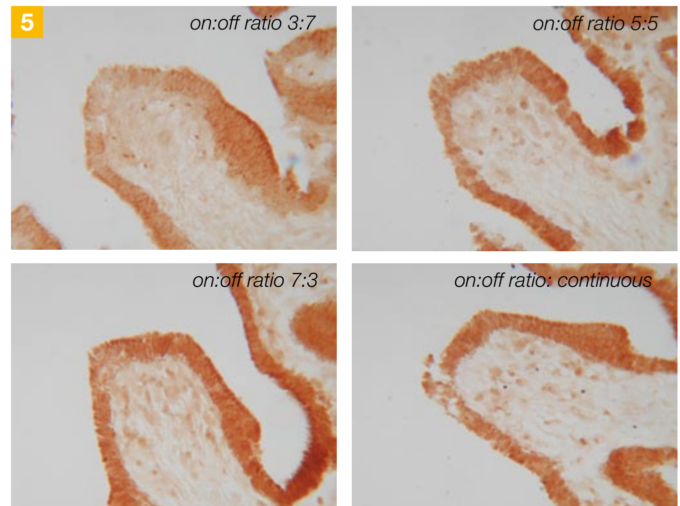


Comparison of different mix power of the SlideBooster

Applying different on/off ratios effects the binding of antibodies and nucleic acid probes.

An on/off ratio series using 3:7, 5:5, 7:3 and continuous mixing was carried out. 7 seconds on-time and 3 seconds off-time showed the highest signal intensities.

These results may vary for each specific system because of different binding constants for different nucleic acid sequences and accessibility of the tissue target region (fig. 5).



Comparison of different on:off ratio of the SlideBooster

NOTE

Nindl I*, Toegl A**, Sterry W*, Stockfleth E* (2004) High sensitivity and reproducibility of immunohistochemistry with microagitation. Arch Dermatol Res 296, p. 278-281

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REFRENCES

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