

Protein Array Protocol

The binding specificity of the purified Atlas antibodies is determined on protein arrays, so called PrEST-arrays, to ensure high specificity toward its antigen and low background binding. The result from the Protein Array analysis of the Atlas antibodies can be viewed on the Human Protein Atlas web portal (proteinatlas.org) on the Antibody/Antigen information page.

Procedure

All washes are performed at room temperature (RT) on a shaker.

1. The PrEST protein (the antigen) is diluted 1:10 in PBS (8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.4) with 100µg/ml BSA (Saveen Werner, Malmö, Sweden) and printed together with 383 other PrEST antigens on epoxy coated microarray slides.

2. The slides are incubated in 37°C over night.

3. Unreacted epoxide groups are blocked by incubating the printed slide in 3% BSA in PBS-T for 60 minutes.

4. The primary antibody is diluted in PBS-T and the dilution is based on the antibody concentration:

Antibody dilution intervals:

for antibody concentrations ≤ 0.04mg/ml, 1:500

for antibody concentrations ≥ 0,05mg/ml, 1:3000

5. 60µl of the primary antibody mixtures are applied to each well and the slide is incubated for 60 minutes. The arrays are separated by a silicon mask (Whatman, Maidstone, UK).

6. The slide is washed 2 x 5 minutes with PBS-T (PBS, 0.1 % (v/v) Tween20) followed by a third wash in PBS for 5 minutes.

7. The secondary antibody mixture is prepared by diluting Goat-anti rabbit Alexa® 647 (Invitrogen, Carlsbad, CA, USA) 60000 fold in PBS-T.

NOTE: *The secondary antibodies are fluorescently labeled and thus light sensitive. The slide should be kept in the dark in the following steps.*

8. The secondary antibody mixture is added to the slide, which is incubated for 60 minutes in the dark.

9. The slide is washed 2 x 5 minutes with PBS-T followed by a third wash in PBS for 5 minutes in the dark.

10. The slide is spin dried for 10-15 seconds using a table centrifuge prior to scanning.

NOTE: *The specified working dilutions of the antibodies are to be considered as a guideline only. Optimal dilutions must be determined by the user.*