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Product Information

Prestige Antibodies®
Powered by ATLAS ANTIBODIES

Protein Array Procedure

Product Description
The binding specificity of the purified Prestige Antibodies® is determined on protein arrays, using PrEST protein fragments, to ensure high specificity toward its antigen and low background binding.

Preparation Instructions
PBS – 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, and 2.7 mM KCl, pH 7.4

PBST – PBS with 0.1% (v/v) TWEEN® 20

Blocking Buffer – PBST with 3% bovine serum albumin

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

Antibody dilution with PBST:
- for antibody concentrations ≤0.04 mg/ml, 1:500
- for antibody concentrations ≥0.05 mg/ml, 1:3,000
  Note: The specified working dilutions of the antibodies are to be considered as guidelines only. Optimal dilutions must be determined by the user.

1. The PrEST protein (the antigen) is diluted 1:10 in PBS containing BSA (100 µg/ml) and printed with 383 other PrEST antigens on epoxy coated microarray slides.

2. The slides are incubated overnight at 37 °C.

3. Unreacted epoxide groups are blocked by incubating the printed slide in Blocking Buffer for 60 minutes.

4. 60 µl of the primary antibody solution are applied to each well and the slide is incubated for 60 minutes. The arrays are separated by a silicon mask.

5. The slide is washed twice, each time for 5 minutes with PBST followed by a third wash with PBS for 5 minutes.

6. The secondary antibody solution is prepared by diluting the goat anti-rabbit IgG – Alexa Fluor® 647 conjugate 60,000-fold in PBST.
   Note: The secondary antibody is fluorescently labeled, and thus, light sensitive. The slide should be kept in the dark during the rest of the procedure.

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

8. The slide is washed twice, each time for 5 minutes with PBST followed by a third wash with PBS for 5 minutes in the dark.

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

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