

Prestige Antibodies™

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ANTIBODIES

Protein Array Procedure

Product Description

The binding specificity of each purified antibody is determined on protein arrays, using PrEST protein fragments, to ensure high specificity toward its antigen and low background binding.

Procedure

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

Antibody dilution:

- 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 (8.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 137 mM NaCl, and 2.7 mM KCl, pH 7.4) and printed with 95 other PrEST antigens on epoxy coated microarray slides.
2. Unreacted epoxide groups are blocked by incubating the printed slide in blocking buffer (~20 ml/slide) for 30 minutes.

3. 60 μl of the primary antibody solution is applied to each well and the slide is incubated for 60 minutes. The arrays are separated by a silicon mask.
4. The slide is washed twice, each time for 5 minutes with PBST (PBS with 0.1% (v/v) TWEEN® 20) followed by a third wash with PBS for 5 minutes.
5. The secondary antibody solution is prepared by diluting the goat anti-rabbit IgG - Alexa 647 conjugate 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.
6. The secondary antibody solution is added to the slide, which is incubated for 60 minutes in the dark.
7. 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.
8. The slide is spin dried for 10–15 seconds using a table centrifuge prior to scanning.

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