

## **FAST™ PAK Protein Array Kit Protocol**

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## I. INTRODUCTION

FAST PAK Protein Array Kits provide the necessary components for researchers who wish to design and build their own protein microarrays, and who are interested in reliability and reproducibility. The kit is based on the FAST Slide surface, a proprietary nitrocellulose-based polymer that offers excellent reproducibility and sensitivity for microarray assays. Unlike other microarray surfaces, FAST Slides have a three-dimensional polymer coating that has been used for decades to immobilize biomolecules.

FAST PAK Kits have been developed with 2-, 8- and 16-pad FAST Slides, for multiple arrays on one slide. These multiple pad formats retain all of the advantages of the original single-pad FAST Slide, with additional benefits. Each pad can be processed separately to increase the number of arrays on each slide, and reduce sample volumes. The multiple pad formats are ideal for multiplexing experiments, side-by-side comparisons, and control experiments all on the same slide.

In addition to FAST Slides, the other key components of FAST PAK are the Protein Arraying Buffer, the Protein Array Wash Buffer and the Protein Array Blocking Buffer. The Protein Arraying Buffer is a proprietary reagent that confers protein stability resulting in increased shelf-life of the array and increased signal compared with other buffers. The Protein Array Blocking Buffer and the Protein Array Wash Buffer both contribute to give superior signal-to-noise ratios on FAST Slides.

FAST PAK has demonstrated sensitivity with antibody arrays down to 1 pg/ml concentration of antigen.

### A. Important Considerations

FAST PAK Protein Arraying Buffer is supplied as a 2x concentrate. This allows the researcher the flexibility of diluting their protein from solution or reconstituting a lyophilized powder to achieve the working 1x solution. It is important to remember that the Protein Arraying Buffer is a 2x concentrate if you make serial dilutions of your protein to be arrayed. The buffer will have to be diluted accordingly.

FAST PAK Protein Array Wash Buffer is supplied as a 10x concentrate. Dilute the 125 ml volume with 1125 ml of dH<sub>2</sub>O to achieve a 1x working solution.

FAST PAK Protein Array Blocking Buffer is supplied as a 1x working solution.

The variability in binding affinities among protein/protein pairs and antibody/antigen pairs combined with the high immobilization capacity of FAST Slides makes it difficult to predict one concentration of protein to array. For initial experiments we recommend arraying a range of concentrations between 0.2 and 2 mg/ml.

### B. Chamber Volumes

The volumes used for blocking, washing and incubation are different for each FAST Slide format. Refer to the chart on the next page for the recommended volumes.

Format	Volume	Format	Volume
1-Pad	700 µl	8-Pad	80 µl
2-Pad	300 µl	16-Pad	80 µl

### C. Kit Components

- 10 FAST Slides
- 25 Incubation Chambers (1- and 2-pad formats only)
- 10 Incubation Chambers (8- and 16-pad formats only)
- 10 ml Protein Arraying Buffer (2x)
- 15 ml Protein Array Blocking Buffer (1x)
- 125 ml Protein Array Wash Buffer (10x)
- Port Seals (1- & 2-pad formats only)
- Chamber Covers (8- and 16-pad formats only)
- Protocol

**NOTE:** *Detection reagents are provided by the user.*

## II. ARRAYING OF PROTEINS

1. Proteins are arrayed in 1x Protein Arraying Buffer. To achieve this, reconstitute lyophilized protein in 1x Protein Arraying Buffer, prepared as above (see "Important Considerations"). If protein is already in a 1X PBS solution, add an equal volume of the supplied 2x Protein Arraying Buffer. Final protein concentration should be between 0.20 and 2.0 mg/ml
2. Array samples onto a dry FAST Slide using an established protocol from a robotic arrayer or manual arraying device like the S&S MicroCaster. FAST Slides are ready for arraying right out of the box. Attention to environmental conditions is critical for accurate spotting. Temperature and humidity must be controlled to avoid evaporation of source plate solution during the array process. Due to the unique nature of FAST Slides and the FAST PAK Protein Arraying Buffer, no activation steps are necessary to immobilize proteins. In order to optimize binding of protein to the surface of the FAST Slide, it is recommended that arrayed slides sit overnight at room temperature.
3. FAST Slides arrayed with antibodies should be stored desiccated, overnight at room temperature in order to maximize the binding activity of the immobilized protein. For some antibodies, drying overnight at 37° C may be advantageous and the drying conditions should be determined for individual proteins.

**NOTE:** *Do not pre-wet FAST Slides. Arraying onto a wet slide will cause the spots to diffuse and is not recommended.*

If using a fluorescent scanner for subsequent detection, the spot diameter should be no more than 250 µm. The array pitch (distance of spots from center to center) can be as little as 300 µm.

## III. BLOCKING THE ARRAY

If the arrayed FAST Slide is not going to be used immediately, store in a cool, low humidity environment (10–30% RH).

When the slide is ready for processing, use an air stream to clean the glass surface surrounding the pads. Carefully remove the backing of an incubation chamber to expose the adhesive, and apply the chamber onto the slide (being careful not to overlap the membrane). Press firmly around the edges to form a secure seal. Add appropriate volume (see step I.B. above) of Protein Array Blocking Buffer into chamber(s). Cover the ports with supplied port seals (1- and 2-pad formats) or cover the chamber with chamber cover (8- and 16-pad formats).

Place the FAST Slide on an orbital shaker or platform rocker for at least 30 minutes, with gentle agitation. Ensure that mixing is occurring by keeping the air pocket inside the chamber in constant motion.

**NOTE:** *If using a blocking buffer other than the Protein Array Blocking Buffer supplied in the kit, do not use BSA or other protein blocking solutions, as this can lead to high background.*

## IV. ADDITION OF ANTIGEN SOLUTION

After blocking, remove port seals or chamber cover and withdraw the Protein Array Blocking Buffer. Add antigen-containing solution (see step I.B. for volumes) into the well(s), and reseal ports, or apply new chamber cover. Incubate one hour to overnight on a rotary shaker.

**NOTE:** *Do not let the FAST Slide pads dry out throughout the remainder of the experiment.*

## V. WASHES

For 1- and 2-pad FAST Slides, remove incubation chamber from the FAST Slide, pour off residual sample and place slide in a dish, tray, or other wash apparatus. Add enough Protein Array Wash Buffer to slightly cover the slide and wash for 5–10 minutes with gentle agitation. Pour off Protein Array Wash Buffer and repeat wash two more times for a total of 3 washes. Multiple FAST Slides can be washed simultaneously in the same dish.

If preferred, 8- and 16-pad FAST Slides can be washed without removing the incubation chamber. Remove the chamber cover and discard the antigen solution by inverting the slide. Fill all wells with Protein Array Wash Buffer and wash three times for 5–10 minutes each.

**NOTE:** *If different antigen solutions were used in different wells, the solutions should be removed with a pipette to avoid cross-contamination.*

If a fluorescently-labeled antigen was used, proceed directly to Imaging, step IX.

## VI. ADDITION OF SECONDARY ANTIBODY

Remove the FAST Slide from the wash apparatus and place on a clean flat surface. Wearing gloves, gently and carefully run a lint-free wipe along the glass edge of the slide, making sure to absorb as much buffer from the glass as possible. Place a fresh incubation chamber (1- and 2-pad formats only) onto the slide, and press firmly around the edges to form a secure seal. Add detection antibody in Protein Array Wash Buffer (see step I.B. for volumes), and seal with port seals or chamber cover. Incubate for 2 hours on a rotary shaker. (The concentration of detection antibody should be determined empirically by the user.)

## VII. WASHES

Remove incubation chamber or chamber cover and residual antibody solution. Wash slide as in step V.

**NOTE:** *If different antigen solutions were used in different wells of the 8- and 16-pad formats, be sure to pipette the solutions out of the wells to avoid cross-contamination.*

## VIII. DETECTION

If a fluorescently labeled secondary antibody was used, proceed directly to Imaging, step IX. If performing a sandwich assay, add the detection reagents as recommended by the supplier, and then repeat wash cycle as in step V.

## IX. IMAGING

For fluorescent detection using a microarray-based imager, the FAST Slide should be dried after the final wash. Air dry the slide until the membrane appears white. Alternatively, slides may be placed in an oven at 80° C for 5 minutes. If heat is used, allow slides to cool for several minutes prior to scanning. Store FAST Slides in a dark, dust-free environment until imaging.

FAST Slides can be detected using a variety of confocal laser scanner systems.

When imaging FAST Slides, the default imager parameters for glass slides will not be suitable for detection. Due to the higher binding capacity of FAST Slides as well as the unique light scattering properties of the polymeric surface, laser power and PMT settings will need to be lower than the settings used for glass slides. The focal length may need to be adjusted due to the thickness of the nitrocellulose polymer (approximately 14 µm) above the glass slide. The laser and PMT settings will also depend on the type of experiment and blockers used.

## Ordering Information

Description	Components	Item #
FAST PAK Protein Array Kit	1-Pad Format (Original)	10 485 262
	2-Pad Format	10 485 319
	8-Pad Format	10 485 322
	16-Pad Format	10 485 325
Please call Technical Service at (800) 245-4024 for information on purchasing FAST PAK components separately.		

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### Further Information

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