Protein Domain Microarray Peptide Screening

1. Use Flexis Robotic Workstation or Accent Microarrayer (Genomic Solution) to spot the proteins on nitrocellulose slides.
2. Block protein microarray slide in blocking buffer (3% milk, 3% BSA, 1X PBS, 0.1% Tween 20) for ~1 hour at room temperature.
3. Label peptides and incubate on ice for 30 minutes:

Phosphorylated Sample	Add	Final [ ]
Specific Peptide (5ug/ul)	2ul	0.5ug/ul
Labeled Streptavidin (100ug/ul)	5ul	25ug/ul
1X PBS	9ul	
Na3VO4 (0.01M)	2ul	1mM
NaF (0.025M)	2ul	2.5mM
Total Volume	20ul	

Non-Phosphorylated Sample	Add	Final [ ]
Specific Peptide (5ug/ul)	2ul	0.5ug/ul
Labeled Streptavidin (100ug/ul)	5ul	25ug/ul
1X PBS	13ul	
Total Volume	20ul	

4. Meanwhile, prepare beads. In two eppendorf tubes, add to each tube 20 ul of biotin agarose beads. Wash once with 500ul of 1XPBST. Keep on ice.
5. Add 500 ul 1X PBST to the peptide+label mix. For phosphorylated samples, add Na3VO4 to 1mM and NaF to 2.5mM final concentration.
6. Add the peptide mix into the first tube of beads and rock for 10 minutes at 4°C.
7. Centrifuge at 14000 rpm for 30 seconds soft stop at room temperature.
8. Transfer the supernatant into the second tube of beads and rock for 10 minutes at room temperature.
9. Centrifuge at 14000 rpm for 30 seconds soft stop at room temperature.
10. Take 500 ul of the supernatant and mix with 1.3 ml of blocking buffer. For phosphorylated samples, add Na3VO4 to 1mM and NaF to 2.5mM final concentration to the blocking buffer.
11. Take the slide from the blocking buffer, but do not dry completely.
12. Transfer the slide into the hybridization chamber and add the labeled peptide probe. Incubate at room temperature for 1 hour OR overnight at 4°C.

13. Wash 3 times with 1X PBST, changing each wash, for 10 minutes each at room temperature. During the third wash, warm up the scanner.
14. Dry the slide at room temperature.
15. Analyze the slide with the Microarray Scanner.